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BLOOD SERUM CHANGES IN THE DOG DURING RECOVERY FROM EXERCISE ON THE TREADMILL[1]

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The present report represents the results of a study of the changes taking place in the blood serum of the dog during the early part of the recovery from muscular exercise. The study was undertaken to provide additional information about processes which are concerned with metabolism in the animal organism during muscular exercise.

Other investigators have reported on various phases of this problem but with man as subject. After short, vigorous exercise blood lactic acid may reach maximum and pH and CO_2 capacity minimum concentrations several minutes after exercise has ended. (Barr and Himwich, 1; Hill, Long and Lupton, 2; Margaria and Talenti, 3; Margaria, Edwards and Dill, 4; and Laug, 5.) According to Margaria, Edwards and Dill (4) the removal of lactic acid after exercise is a slow process, one-half being removed in fifteen minutes, but Newman, Dill, Edwards and Webster (6) have shown that the rate of removal is greatly increased if the hard work which has produced the lactic acid is followed by more moderate work rather than by complete rest. According to Laug (5) the total CO_2 of the blood continues to fall steadily after the lactic acid concentration has started to fall and the pH to rise, possibly a compensation for excretion of base in combination with lactic acid by the kidneys.

Havard and Reay (7) have found that the inorganic phosphate concentration of the blood falls rapidly below the normal level after exercise has ceased. The decrease in concentration is greater in untrained subjects

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and after longer periods of vigorous exercise. Similar observations have been reported by Riabouschinsky (8) and by Gemmill and Ribeiro (9).

Keys (10) has shown that the recovery of fluid by the blood is rapid during the first minute of rest after brief, severe exercise but slow for the next 30 to 40 minutes. While the potassium concentration (11) may be as much as 25 per cent above the initial level immediately after work, it drops precipitously to 5 to 15 per cent below the initial level in the next 10 to 15 minutes, rises to 20 per cent above that level in the next 40 minutes and regains its initial value in 1 to $1\frac{1}{2}$ hours. Except for the initial rise, adrenaline produces the same effect; however, while injection of adrenaline causes a marked fall in the potassium level of the plasma in man, it produces the reverse effect in the dog, a marked rise in the potassium concentration of the plasma (12). Keys found the sodium concentration of the plasma 2 to 10 per cent above the resting level after one minute of violent exercise, but at the resting level 15 minutes later (11).

Experimental procedure. Five short-haired male dogs, weighing 9 to 20 kgm. have been used in the experiments. Exercise consisted in running on a motor-driven treadmill set at either a 15 or 20 per cent incline and at a rate of 5 or 6 miles an hour. Three series of experiments were carried out. In the first series the dog ran very fast for 2 minutes and was taken off the treadmill before his first energetic sprint had slowed down. In the second and third series the dog ran longer, approximately 25 to 37 minutes in the second series, 55 to 72 minutes in the third, and was taken off when he began to tire as evidenced by lagging against the back of the mill. Fatigue set in earlier in the second series than in the third for various reasons. In some cases the treadmill was set at a higher incline or a faster rate, in others the dog was not so well trained, and in still others the innate capacity of the individual dog was less. One dog participated in all three series.

Venous blood samples were drawn under oil before exercise, immediately after (approximately 2 minutes were required for drawing the sample), and at intervals during recovery. In the first series an attempt was made to draw samples at as frequent intervals as possible during the first 10 minutes of recovery as well as after 30 and 120 minutes. The results of analysis on these samples were plotted against the time of drawing the sample and values interpolated for 3, 6 and 10 minutes after exercise in order to average the results for a given dog. In the second and third series samples were drawn at approximately 3, 10, 30 and 120 minutes after the dog stopped running.

The serum of each sample was separated from the cells under oil and analyzed for pH, total fixed base or sodium, total solids, protein and potassium. Most of the analytical methods have been described in earlier publications (13, 14, 15). In later experiments the silver nitrate-thio-

cyanate method for chloride has been replaced by the silver iodate-thiosulfate method of Sendroy (16). For potassium the chloroplatinic acid method of Shohl and Bennett (17) was used, with thiosulfate titration of the potassium iodoplatinate formed by the reaction of the chloroplatinate with potassium iodide, but with certain modifications suggested by Hald (18) and Hartzler (19).

Discussion of results. The results are shown graphically in figures 1, 2, 3. Each curve represents an average of two or three experiments on a given dog. The change in value from the initial concentration for each serum component studied is plotted against minutes after exercise. The horizontal line represents the initial level in each case. That portion of the curve to the left of the vertical line represents the extent of the change in each component from the initial resting state to the end of the exercise period, while the distance from the initial point to the vertical line represents the length of the exercise period.

The rise in pH characteristic of overventilation in the dog is higher in the second series of experiments in which difficult work is performed for 25 to 37 minutes. Under these conditions the return to the initial pH after exercise is seldom complete within 30 minutes. The 2 minute exercise period of the first series is apparently not long enough to produce the maximum effects of overventilation. In the third series the work is less difficult for the dog and the effects of overventilation are not so evident. In the first and third series recovery to normal pH is usually complete within 10 minutes.

The curves show practically the same rise in serum lactate at the end of both the 2 minute and the 25 to 37 minute run, but a marked delay in recovery in the longer run as contrasted with a rapid recovery after the two minute run. This lag in recovery may indicate a greater degree of fatigue after the longer run.

The further rise in lactate which occurs in many instances in the first few minutes after the 25 to 37 minute run would seem to be significant, especially since it does not occur after the 2 minute run. This finding suggests that simple diffusion of lactate ions from a higher concentration in the tissues into the blood is inadequate to account for the increased lactate concentration of the blood after the longer run. The increased anaerobic conditions of the very short run caused by its more vigorous nature and the lack of time for sufficient cardiovascular adjustments to meet the oxygen needs would lead presumably to a faster accumulation of lactic acid in the tissues and a higher concentration gradient between the tissues and the blood. The fact that the lactate of the blood under these conditions does not increase appreciably after exercise ceased although it increases significantly after the 25 to 37 minute run suggests that the greater degree of fatigue after the longer run prevents the rapid

removal of lactic acid either from the tissues by oxidation or resynthesis, or from the blood by such reactive centers as the liver—processes which,

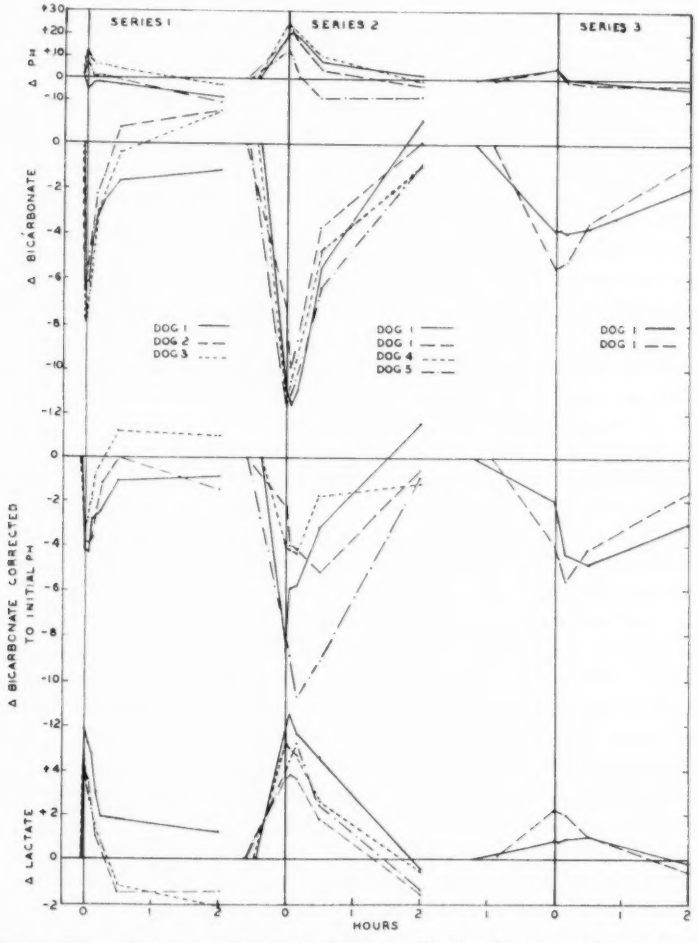

Fig. 1. Changes in concentrations of serum pH, bicarbonate and lactate during recovery from exercise. Bicarbonate and lactate concentrations are expressed as milli-equivalents per kilogram serum H_2O.

in the short run, may occur so rapidly that they mask any diffusion of lactate from the tissues to the blood.

The bicarbonate curves follow inversely the lactate curves. The fall in bicarbonate usually exceeds the rise in lactate by 2 to 3 m.eq. The

bicarbonate concentrations after correction to initial pH by means of the relation $\frac{\Delta[HCO_3]_s}{\Delta pH_s} = -28$ (20), show less rather than greater resemblance

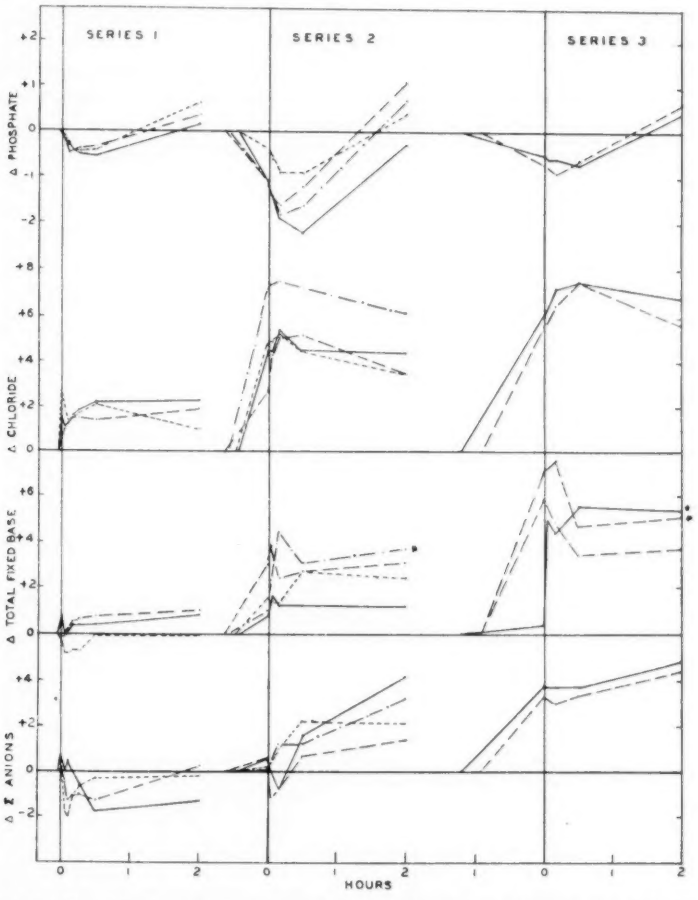

Fig. 2. Changes in concentrations of serum phosphate, chloride, total fixed base and total determined anions during recovery from exercise. Concentrations are expressed as milli-equivalents per kilogram serum H_2O. The asterisk indicates sodium concentrations rather than total fixed base.

to the lactate curves and suggest that other factors are involved. During the first few minutes of recovery there often occurs a significant fall in serum bicarbonate which appears unrelated to the small increase in lactate

concentration which may also occur, since it exceeds it in amount and persists. Laug (5) observed the same phenomenon in similar experiments on man and suggested that it might be a compensation for excretion of base in combination with lactic acid by the kidneys. In the present experiments a disproportionate rise of chloride as compared with total fixed base supports a similar interpretation.

Figure 2 shows a rise in serum chloride, not only during exercise, but also for some minutes afterwards. The fact that the extent of the chloride

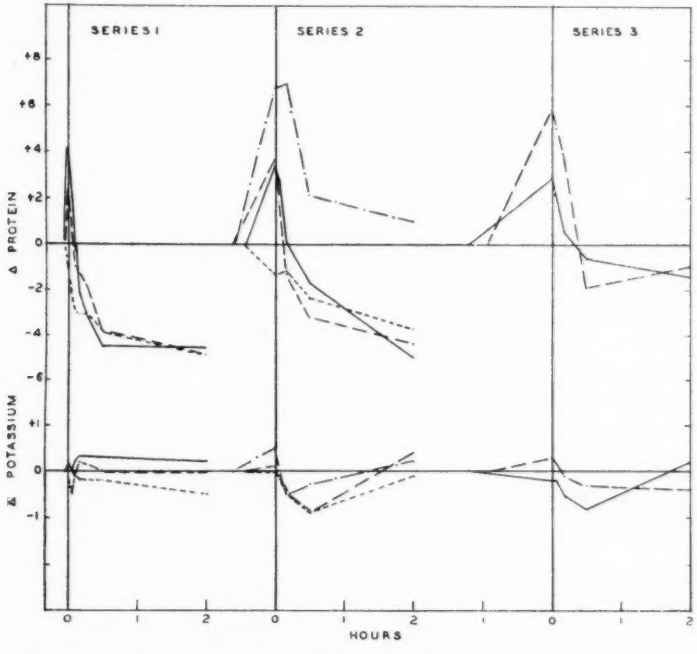

Fig. 3. Changes in concentrations of serum protein and potassium during recovery from exercise. Protein concentrations are expressed as grams, potassium concentrations as milli-equivalents per kilogram serum H_2O.

rise during exercise varies approximately according to the length of the run suggests that the rise in chloride is due chiefly to a concentration of body fluids through loss of water by panting. Since panting continues for some minutes after exercise ceases, the rise in chloride at that time may simply reflect the dehydrating process. The rise in chloride after exercise cannot be associated with a pH change since it is opposed in direction to the chloride-bicarbonate shift which would be anticipated from the return of serum pH to the initial level during recovery.

The changes in the inorganic phosphate concentration of the serum of the dog during recovery agree with those observed in man, an abrupt fall during the first 10 minutes of recovery to a low level which may be maintained for some time and a return to the initial level or above by the end of two hours of rest. The constancy of this finding emphasizes the importance of phosphate in the recovery process and gives added support to the suggestion of Havard and Reay (7) that phosphate is being extracted from the blood by the tissues for increased resynthesis of hexosephosphate.

The serum potassium curves during recovery bear some resemblance to the inorganic phosphate curves for they show a marked fall during the first half-hour of recovery followed by a return to the initial level two hours later. The similarity of these curves suggests that potassium in the form of the potassium salt of phosphoric acid is also being utilized by the tissues in the resynthesis of glycogen during recovery.

Changes in the serum protein concentration during exercise and recovery reflect changes in the fluid content of the plasma, the flow of fluid out of the plasma into the tissue spaces during exercise which occurs in the majority of dogs in response to hydrostatic or osmotic forces, the rapid return of fluid into the blood when exercise ceases, and the further dilution of the blood with fluid from the tissue spaces in response to loss of blood by sampling. In only one instance is there evidence of a delayed return to normal conditions after exercise.

We have attempted to compare the changes in the sum total of determined anions of the serum with those in total fixed base. Unfortunately, in spite of the care taken in the analyses, the experimental errors in both are large compared with the changes observed and the results have little value except to indicate the degree to which the two check each other and to emphasize the impossibility of discerning small changes by a method as unsatisfactory as that used for total fixed base.

SUMMARY

The changes in the electrolyte pattern of the blood serum during recovery from muscular exercise on an inclined treadmill have been studied in five dogs. The experiments may be grouped into three series according to the type of exercise: *a*, 2 minutes of very rapid running; *b*, 25 to 37 minutes of vigorous running to the point of fatigue, and *c*, 55 to 72 minutes of less vigorous running to the point of fatigue.

The results may be summarized as follows. Serum lactate often continues to rise and bicarbonate to fall for some minutes after exercise, but the fall in bicarbonate after exercise exceeds the rise in lactate. Although similar high lactate concentrations are reached after both the 2 minute and the 25 to 37 minute vigorous run, the return to the initial concentration is more rapid after the shorter run. Inorganic phosphate

and potassium fall markedly for some time after exercise but usually return to the initial level within 2 hours. Serum chloride often continues to rise for at least 10 minutes after exercise and remains elevated above the initial level two hours later, but the concentration of serum protein, with few exceptions, falls rapidly below the initial level after exercise and fails to return.

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THE EFFECTS OF FORMALIN ON THE HORMONES OF HUMAN PREGNANCY URINE[1]

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In former investigations we have shown that by subjecting a single anterior hypophysis of cattle to the action of formalin (Mallinckrodt's formaldehyde solution, analytical reagent) in vitro and subsequently implanting one-fourth of such a gland on four successive days into a guinea pig, a definite series of changes can be obtained in the ovary, which are in accordance with the strength of the formalin solution used and the length of time during which it was allowed to act (1, 2). The first stage was characterized by a slight reduction of the luteinizing effects; the next, by the production of fully mature follicles in addition to the diminished luteinization. When the optimum concentration was reached the luteinizing effects were largely suppressed, although a slight remnant could still be present. The changes in the secondary sex organs corresponded to the ovarian effects.

Early in 1936 we extended these experiments to pregnancy urine and found that, in principle, formalin exerts here the same sequence of effects. There exist, however, differences in the effective doses for cattle gland and for pregnancy urine. Moreover it could be shown that in pregnancy urines obtained from different individuals, three to three and a half months after the beginning of pregnancy, the effective doses differ within a certain range. In a number of experiments, instead of pregnancy urine we injected suspensions of pregnancy urine powder, either untreated or treated previously with formalin. For the production of the powder 1000 cc. of urine were slightly acidified with acetic acid and a precipitate was produced by the addition of four volumes of 95 per cent alcohol; this precipitate was suspended first in 500 cc. of 80 per cent alcohol and subsequently in 500 cc. of ether. The follicular hormone remained in the solution and the powder was free from estrogen. The yield from 1000 cc. of urine was 3.75

[1] These investigations were carried out with the aid of grants from the International Cancer Research Foundation, from the Committee on Research in Endocrinology of the National Research Council, and from the Jane Coffin Childs Memorial Fund for Medical Research.

grams of dry powder. For injection 10 mgm. of dry powder were suspended in 1 cc. 0.9 per cent NaCl solution and this suspension was injected intraperitoneally.

In addition to the effects of formalin-treated pregnancy urine, we again studied in control experiments the effects of untreated pregnancy urine on guinea pigs, and also the effects of normal untreated and formalin-treated urine of women obtained during various stages of the menstrual period. Altogether 292 guinea pigs were used in these investigations, including 30 animals in which the effect of normal urine was tested with or without the addition of formalin.

Our observations concerning the action of normal pregnancy urine tend to confirm our previous conclusion (1, 3), that the luteinizing changes in theca interna and interstitial gland, the premature enlargement of granulosa, and the various combinations of both these changes, exert an inhibiting effect on the formation of fully mature follicles, and furthermore, that the first mentioned changes inhibit estrogenic effects on the vagina. These various changes in the ovary of the guinea pig, other than the development of fully mature follicles, take the place of the formation of true corpora lutea and pseudocorpora lutea in rabbit, rat and mouse.

In each experiment the urine was divided into two portions: the first portion was kept in the refrigerator for injection into control guinea pigs, while to a second portion formalin was added. Concentrations, varying between 0.25 per cent and 4 per cent, of this latter substance were used in different experiments. In the majority of cases the mixtures of formalin and pregnancy or normal urine were kept at a temperature of 37° or 39°C. and at pH 8 for a period of seven days; in other experiments the period was three or four days, and in still others the mixtures of urine and formalin, after remaining for seven days at 37°, were kept for variable lengths of time at room temperature or in the refrigerator at about 4°C. Guinea pigs were used as the test animals in all these experiments. In the majority of instances 1 cc. of the above substances was injected intraperitoneally on four successive days, but there were some variations in the quantity of material injected as well as in the number of days during which the injections were given.

In table 1 the results obtained in our experiments are summarized. Those reactions are positive in which fully mature follicles are produced and in which the secondary sex organs show the estrous effects. The experiments are negative if fully mature follicles are lacking in the ovaries and the secondary sex organs are resting or show changes indicating a weak stimulation. Reactions are considered as intermediate in cases in which the production of typical fully mature follicles was prevented by a premature growth of connective tissue into the granulosa during the process of maturation and in which the effects on the sex organs are intermediate.

We may conclude from table 1 that with a concentration of 0.2 per cent to 0.3 per cent some positive reactions occur, but the negative results greatly predominate. With a concentration of 0.5 per cent the negative results still predominate, but less than with the lower concentration. With 0.75 per cent the positive results begin to predominate. From this concentration on to a concentration of 3 per cent is the optimal range. However, even here a considerable number of negative reactions still occur; furthermore, in the pregnancy urines obtained from different individuals the optimal concentration of formalin differs. Whereas the optimal concentration of formalin when acting on pituitary glands of cattle is about 0.25 per cent, it is much higher in the case of pregnancy urine; here it varies between 0.75 per cent to 3 per cent. Even a 4 per cent concentration of formalin in pregnancy urine may still be effective. This difference

TABLE 1

Effect of concentrations of formalin on the hormone of human pregnancy urine

PREGNANCY URINE CONCENTRATION OF FORMALIN	REACTION IN OVARY		
	Positive	Intermediate	Negative
per cent			
0.2, 0.25, 0.3	2	2	9
0.5	17	0	22
0.75	17	5	9
1.0, 1.5	34	2	17
2.0, 2.5	16	2	9
3.0	11		6
4.0	2		
PREGNANCY URINE POWDER			
0.2, 0.25, 0.3	6		8
0.5	0		4

between the action of formalin on pituitary glands and on pregnancy urine may be expected if we consider that the solid gland tissue may absorb and accumulate the formalin, while this is not the case in the fluid pregnancy urine. Correspondingly, we should expect the optimal concentration of formalin with pregnancy urine powder to be similar to that of pituitary glands. This seems at least to be indicated by the experiments which we have made so far, although the number of the latter is too small at present for a definite conclusion. With pregnancy urine powder the optimal concentration ranged between 0.2 per cent to 0.3 per cent.

If the pregnancy urine to which 0.5 per cent or 0.75 per cent formalin has been added is incubated for only one or three days instead of for seven days, the results are negative; under these conditions the action of formalized pregnancy urine is too weak to induce full maturation in the ovary

and estrus in the secondary sex organs. If pregnancy urine was exposed to formalin action for longer than seven days at 37°C. positive results were obtained in a number of instances. Positive results were obtained in the following cases: with a 0.5 per cent formalin pregnancy urine mixture which had been kept first for seven days at 37°C., and subsequently for seven months at room teperature; with a 1 per cent formalin pregnancy urine mixture kept for seven days at 37°C., and for thirty or thirty-five days at 4°C.; with a 2 per cent and 3 per cent formalin pregnancy urine mixture kept for seven days at 37°C., and subsequently for from thirty to ninety-one days at room temperature. It is therefore possible to preserve the full maturation and estrus-producing effect of formalized pregnancy urine for as long as three to seven months, provided the concentration of formalin chosen was suitable for the particular kind of pregnancy urine. Similar

TABLE 2*

Differences in the effective concentration of formalin in different pregnancy urines

	0.5 PER CENT	0.75 PER CENT	1 PER CENT	1.5 PER CENT	2 PER CENT	3 PER CENT	4 PER CENT
Urine A		−−	+−		+−	+−	
Urine B		++	++		++	+−	
Urine C		−−	++		+++	++	
Urine D		+−	++		++−	+−	
Urine V	++−						
Urine IX	−−−−−−−		−−−				
Urine XII	++		+++++−	++			
Urine XIII	−		++	−			
Urine XIV	−−−		−−−−	−−−−	+−−−	+−	++
Urine XV			++	−	−−		
Urine XVI			−	−−	++	++	

* Each symbol (+ or −) in the above table represents the result of one experiment.

results were previously obtained with pituitary glands kept in vitro in suitable concentrations of formalin solutions.

The concentrations of formalin which cause full maturation of follicles and estrous changes in uterus, vagina and mammary gland differ in the pregnancy urines of different individuals.

In the case of pregnancy urines C, XV, and XVI, the optimum concentration of formalin ranged between 2 per cent to 4 per cent, but a 1 per cent concentration was also effective. With pregnancy urines B, V, and XII, low concentrations of formalin, varying between 0.5 per cent to 1.5 per cent, were effective, but urine B was also active at a 2 per cent, and partially active at a 3 per cent concentration. Other urines were intermediate between these two types.

These differences might be interpreted as indicating that either the

absolute amount of luteinizing hormone or the ratio between luteinizing and maturation hormones differs in different urine. The more the luteinizing hormone preponderates, the greater will be the concentration of formalin required to produce full maturation and estrus effects.

Normal pregnancy urine produced various processes of luteinization in the ovary of the guinea pig, such as luteinization of the theca interna of atretic follicles and also of preserved follicles, formation of interstitial gland, production of interstitial gland bodies, and luteinizing connective tissue atretic follicles, premature enlargement of granulosa, and formation of pseudolutein bodies.[2] Treatment of the pregnancy urine with formalin reduces or entirely suppresses these changes; it makes possible thereby the full maturation of large follicles. In an approximate manner we may distinguish different degrees in the action of formalin on pregnancy urine: *Stage I.* The concentration of formalin is so weak that the changes produced in the ovaries are those of pregnancy urine. *Stage II.* While the various luteinization processes are somewhat diminished, they still remain quite noticeable; fully mature follicles have not yet developed. The premature enlargement of granulosa in small follicles and the luteinizing connective tissue atresia disappear first. *Stage III.* Side by side with the various luteinizing processes in theca, including formation of interstitial gland and the production of pseudolutein bodies, fully mature follicles appear, which have, however, the tendency to become readily transformed into pseudolutein bodies through ingrowth of connective tissue and vessels into the granulosa. *Stage IV.* The luteinizing processes are very much reduced or absent almost entirely and fully mature follicles, which now appear, have no longer a tendency to become so readily converted into pseudolutein bodies and pseudocorpora lutea. *Stage V.* In the last stage the formalin action on the pregnancy urine is so strong that not only the various luteinizing processes are eliminated, but also the maturation of follicles. The injection of the formalized pregnancy urine no longer affects very noticeably the ordinary structure of the ovary. While it is thus possible to distinguish different degrees of intensity in the action of formalin on pregnancy urine, the various stages thus established in the efficacy of formalized pregnancy urine do not correspond to absolute quantities of formalin owing to the fact mentioned above that different pregnancy urines differ in the concentration of the gonadotropic hormones.

As to the condition of the secondary sex organs, the presence of fully mature follicles in the ovaries was usually associated with the production of squamous epithelium and keratin in the vagina, although in some cases keratinization had not yet occurred at this stage. The uterus showed

[2] As to the terms used in describing the various luteal changes in the ovary of the guinea pig see reference 1c.

estrous changes and the mammary gland was actively proliferating. In experiments which did not lead to the development of fully mature follicles, but in which the various luteinizing structures were present, there was usually a slight proliferation of the epithelium of the vagina. In the uterus a mild mitotic proliferation in connective tissue and blood capillaries was found in a number of instances; in the mammary gland proliferation was entirely or almost entirely lacking. On the whole we find, therefore, under these conditions a state of slight stimulation.

After a single injection of 1 or 2 cc. of pregnancy urine into guinea pigs, atresia of all except the smallest ovarian follicles usually sets in; a similar effect was seen after injection on two successive days. After injections over a period of three and four days a gradual recovery was observed to take place in many cases, notwithstanding the continued injections, and follicles grew again to a larger size. In this respect the effects of pregnancy urine differed from those seen after injections of pituitary extracts, in which under otherwise similar conditions the atresia persisted for a longer period of time. If the injections of pregnancy urine were interrupted for a few days the follicles resumed their full growth. Treatment of pregnancy urine with formalin did not necessarily abolish the atresin action, but it seemed to diminish it, at least in many instances. After injection of normal urine distinct atresin effects were not observed.

Discussion. It follows from these experiments that formalin exerts the same effects on the hormones of human pregnancy urine which it exerts on the pituitary gland of cattle. In both cases it diminishes or suppresses a number of changes which these two substances otherwise exert on the ovary of the immature guinea pig; instead, it makes possible the development of fully mature follicles and the typical estrogenic changes in the secondary sex organs. If the action of formalin is too strong, all the effects of the hormones of the pituitary or pregnancy urine disappear. We may therefore conclude that formalin affects in an identical manner the hormones of the pituitary gland and of pregnancy urine; the only difference seen consists in the concentrations necessary to obtain these results.

Considerable doubt exists as to the relation of the hormones of the pituitary gland and of pregnancy urine to each other; in this connection it seems therefore that the data here recorded as to the identity of the effects of formalin, and especially of the sequence of effects in accordance with variations in the concentrations of formalin, on the hormone actions of both the pituitary gland and the pregnancy urine are of very great interest.

As to the difference in the action of human pregnancy urine and cattle pituitary on the ovary of the guinea pigs, this could be explained by the assumption that in the pituitary gland of cattle the maturation hormone preponderates over the luteinizing hormone to a greater extent than in human pregnancy urine. Implantation of cattle pituitary gland might,

therefore, be expected to be more effective in causing growth and maturation of follicles than injection of pregnancy urine, which increases the tendency to theca luteinization, premature enlargement of granulosa, and early ingrowth of connective tissue and vessels into the granulosa, and which thus makes difficult the growth and full maturation of follicles. In hypophysectomized animals the absence of growth stimuli, which normally originate in the animal's own hypophysis and cause the granulosa to grow and mature, would have additional effects. In this way it may be possible to explain the lack of follicular maturation after injection of pregnancy urine into hypophysectomized rats, and it would then not be necessary to assume that the hormones of pregnancy urine are different from those present in the pituitary of cattle.

It is the change in the structure of the granulosa cells, which is characteristic of mature follicles, rather than the luteinization of the theca cells which is so common a change in the ovary treated with gonadotropic substances and which is not associated with fully developed estrogenic effects. It is therefore probable that it is primarily the changes in the granulosa which are responsible for the sudden increase in the production of estrogenic substance in the ovaries rather than the change in the theca interna at the time when fully mature follicles appear.

In earlier experiments Bischoff and Long (4) found that if human pregnancy urine is freed from estrogen by treatment with alcohol and ether, formaldehyde leaves prolan either unchanged or it destroys it if the concentration of formaldehyde exceeds a certain degree; immature rats were used in these experiments and the action of formalin on the weights of the ovaries as well as the presence or absence of corpora lutea served as criteria for the effects on prolan. In a paper of which we learned only after conclusion of our experiments, Brindeau, Hinglais and Hinglais (5) state that 1 per cent of commercial formol leaves prolan A of human pregnancy urine, from which estrogen has been removed, unchanged after the formalized pregnancy urine has been kept on ice for fifteen days; but prolan A decreases after eighty days. Formol is therefore practically without effect on prolan A. Prolan B, on the contrary, is gradually destroyed; there exists therefore a sharp distinction between prolan A and prolan B. These experiments were presumably carried out on rats, but the methods used for testing the effectiveness of prolan are not stated. The conclusions of these investigators differ from our conclusions, based on experiments in the guinea pig, in which we found quantitative differences in the action of formalin on the gonadotropic hormones of pregnancy urine.

SUMMARY

The effects of formalin on human pregnancy urine differ in accordance with the concentrations of this substance which are used. If the effects of pregnancy urine on the ovary of the guinea pig are used as indicators,

the following grades of action of formalin on pregnancy urine can be recognized: Grade I,—diminution in theca luteinization and accelerated enlargement of the granulosa of large follicles, combined with early ingrowth of connective tissue and capillaries into the granulosa of not yet fully mature follicles. Grade II,—production of fully mature follicles accompanied by diminution of the luteal changes. Grade III,—development of fully mature follicles and complete or almost complete suppression of theca luteinization as well as of the premature enlargement of granulosa and invasion of granulosa by connective tissue and capillaries. Grade IV,—destruction of maturation effects. Estrous changes in vagina, uterus and mammary gland accompany the production of fully mature follicles.

We may conclude that formalin inhibits, in the first place, the luteinizing effects of human pregnancy urine and if used in stronger concentration it destroys also the follicular maturation effects. The action of formalin on both these hormones differs, therefore, merely in a quantitative manner. Different pregnancy urines differ in the concentration of formalin needed to accomplish these effects; this fact is probably due to differences in the concentrations of these two hormones in pregnancy urine. It is possible to preserve for a long time pregnancy urine modified by formalin in such a way that it produces mainly maturation and estrogenic effects in the ovary of the guinea pig. It is significant that formalin acts in an identical manner on the hormones of cattle pituitary glands and of pregnancy urine.

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PULMONARY ARTERIAL PRESSURE IN EXPERIMENTAL RENAL HYPERTENSION

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Although no actual evidence exists to indicate the presence of a humoral mediator in renal hypertension, the experimental evidence favors this view (1, 2). It therefore seemed to us that a study of the properties of the humoral mediator responsible for the rise in systemic arterial pressure was essential. In this connection, it seemed desirable to determine if the chemical mediator of systemic arterial hypertension also caused a pulmonary hypertension.

METHOD. For this purpose, direct observations were made of the simultaneous pulmonary and femoral arterial pressures with the Hamilton technique (3) before and after the induction of experimental renal hypertention using the Goldblatt technique (4). Two types of experiments were employed.

In the first the pressure measurements were made, at the appropriate times, in dogs anesthetized with nembutal (25 mgm. per kilo). After artificial respiration was established, the chest was opened under aseptic precautions in the left third or fourth intercostal space, the opening enlarged by retractors, and the pulmonary artery brought into view. The pericardium was then opened and the pulmonary artery and femoral artery were simultaneously needled for the pressure determinations. The pericardium and the chest were then closed, the pneumothorax relieved and the animal permitted to recover.

In the second method the observations were made on *trained* unanesthetized dogs in which a silver cannula had previously been attached to the pulmonary artery and brought under the skin, in a manner similar to the methods suggested by London (5), de Burgh Daly (6), and Hamilton, Woodbury and Vogt (7).

Difficulties were encountered in obtaining survival of such pulmonary cannulated animals and as a consequence certain precautions were developed to counteract the adverse influences. The final method of cannulation and the precautions used merit a brief description.

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Medium sized dogs (21 kilo) were used. The animals were anesthetized with nembutal intravenously (25 mgm. per kilo), placed on their back with the left forelimb fastened above the head. The left chest wall was carefully shaved and thoroughly cleansed with soap and water, followed in turn by benzene, alcohol, and a 5 per cent alcoholic iodine solution. The animal was then draped and the operation done under *strict* asepsis. This was found essential to avoid chest infections. An incision 4 cm. in length, was made through the subcutaneous tissue to the fascia lying over the muscles along the third or fourth intercostal space, starting 2 to 3 cm. from the left parasternal line. This fascia was cut at right angles and the exposed muscles were then separated in the direction of the fibers. This ensured good closure later. The intercostal muscles, however, were cut across and the parietal pleura exposed and incised. Artificial respiration was started the moment the pleural cavity was opened.[2]

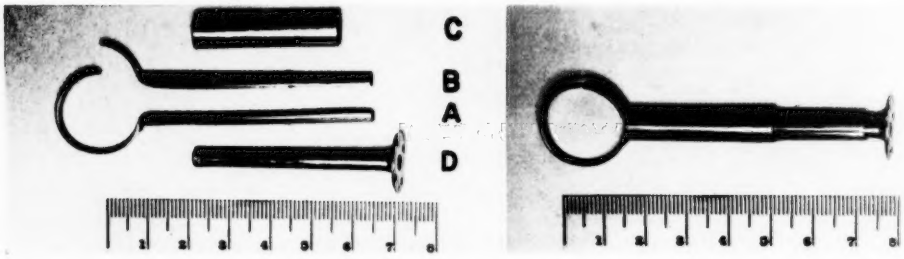

Fig. 1. Photograph on the left shows the four parts of the final type of silver pulmonary cannula used. The photograph on the right shows the cannula assembled. Scales in cm. Described in text.

The opening into the pleural cavity was enlarged by means of an adjustable retractor. The lungs were covered with towel strips moistened with isotonic saline solution and pushed to either side and the pericardium over the pulmonary artery was opened and the special cannula applied to the latter.

Two types of silver pulmonary cannulae were used. The first was similar to that used by Hamilton et al. (7). The second was a modification which was found to be more satisfactory.

The longer curved limb (*A*, fig. 1) of the inner section of the second type

[2] A soft rubber tube introduced into the trachea and connected with a pump was used for artificial respiration. A bottle containing glass beads and water, kept in a warm water bath, was present between the pump and the tracheal tube to ensure that the air entering the lungs was warm and moist. It also acted to smooth out the pressure fluctuations during the respiratory cycle. Before entering the tracheal tube the air was filtered through cotton.

of cannula was passed around the pulmonary artery by introducing it between the walls of the aorta and pulmonary artery. The shorter curved limb (*B*) was then applied and the cylinder (*C*) passed over both to hold them in place. A ridge on the two curved limbs prevented this cylinder from slipping off. The chest piece (*D*) was inserted through the various layers of tissue in the same or preferably a higher or lower intercostal space than the one with the chest opening, in such a way that it would fit within the inner ensemble of the cannula without kinking the pulmonary artery. The length of the fitted cannula was adjusted to the distance between the pulmonary artery and chest wall. The inner and outer parts of the cannula were fitted snugly together so that they did not slide easily but permitted some motion. The overlap of the two parts was such as to avoid separation when the animal took an excessively deep inspiration. Once the cannula pieces were properly adjusted, the incision was carefully closed layer by layer. Before the skin layers were closed the outer part of the cannula was sewed to the subcutaneous layer with silk, and the pneumothorax was relieved, the wound aseptically bandaged and the animal allowed to recover.

Certain postoperative precautions were found to be vital for success: 1. The period of anesthesia was shortened by use of metrazol (3 cc.) or coramine (3 cc.) intravenously. 2. About 300 cc. of 5 per cent dextrose in isotonic Ringer solution was injected subcutaneously. 3. The animals were kept warm with covers and radiant heat. With these procedures, the dogs were found to be alert and able to drink water 18 hours after the operation and the pulse was usually normal in rate by the third day. 4. Prontosil (10 cc.) followed by sulfapyridine (0.5 to 1 gram daily) was used routinely.

After recovery the animals were trained for femoral pressure determinations (2, 8). Following the establishment of a control blood pressure level, the animal was subjected to simultaneous femoral and pulmonary artery pressure determinations. This could be done without upsetting the trained animal if about 2 cc. of 1 per cent procaine HCl solution was injected intra- and subcutaneously over the site of skin puncture in the chest. After simultaneous readings were obtained the animal was subjected to renal artery clamping and repeated readings were taken at various periods during the ensuing hypertension.

Discussion of results. *Normal pulmonary pressure level.* Tables 1 and 2 show that the normal pulmonary pressure levels were similar in the open chested anesthetized dog and in the normal trained unanesthetized dog. Typical records of the latter are shown in figure 2.

The values obtained by us on unanesthetized *trained* dogs probably represent the true normal pressure in the central pulmonary artery. These values range from 35–25/15–7 mm. Hg and average 28/11 mm. Hg. The

TABLE 1

Blood pressure in dogs anesthetized with nembutal, having artificial respiration and opened chest

DOG NUMBER	TIME IN RELATION TO ONSET OF SYSTEMIC HYPERTENSION	CENTRAL PULMONARY ARTERIAL PRESSURE		FEMORAL ARTERIAL PRESSURE	
		*mm. Hg**		*mm. Hg**	
1	Before	25/14		140/80	
2	Before	28/16		160/115	
3	Before	30/15		175/100	
	9 days after		25/15		225/125
	99 days after		21/8		195/135
4	Before	25/16		170/105	
	26 days after		27/13		200/130
Average with normotensive systemic pressure		27/15		160/100	
Average with hypertensive systemic pressure			24/12		207/130

* Systolic/diastolic.

TABLE 2

Blood pressure in unanesthetized trained dogs with pulmonary cannula

DOG NUMBER	TIME IN RELATION TO ONSET OF SYSTEMIC HYPERTENSION	CENTRAL PULMONARY ARTERIAL PRESSURE		FEMORAL ARTERIAL PRESSURE	
		mm. Hg§		*mm. Hg§*	
1	Before	25/15		150/75	
2	Before	30/12		125/80	
3	Before	25/15		140/80	
4	Before	25/15		140/80	
5	Before	35/7		130/60	
6	Before	35/10		115/65	
7	Before	25/10		150/85	
	1 day after		23/5		160/100
	11 days after‡		21/7		230/125
8	Before	30/8		160/80	
	1 day after*		37/7		225/100
	3 days after		27/7		250/140
	5 days after		28/18		240/125
	9 days after†		32/7		170/100
Average with normotensive systemic pressure		28/11		139/75	
Average with hypertensive systemic pressure			28/9		212/115

* Pulsus bigeminus present.

† Developing uremia at this time—blood NPN 225 mgm. per cent, NPN previously normal.

‡ Dog died suddenly from pericardial tamponade after hemopericardium following perforation of pulmonary artery by needle.

§ Systolic/diastolic.

systemic blood pressure levels in our dogs had fulfilled the criteria previously established by us (2, 8) indicating that the animals had not been disturbed by the procedure. The systemic arterial pressures in each of the 8 dogs studied were well within the normal range at the time the pulmonary pressure reading was made.

The higher pulmonary values reported by others are probably the result of disturbances in the balance of the output of the right and left heart, the result either of excitement in *untrained* unanesthetized dogs (6, 7) or of relative left heart failure in the anesthetized animals (9, 10, 11). In favor

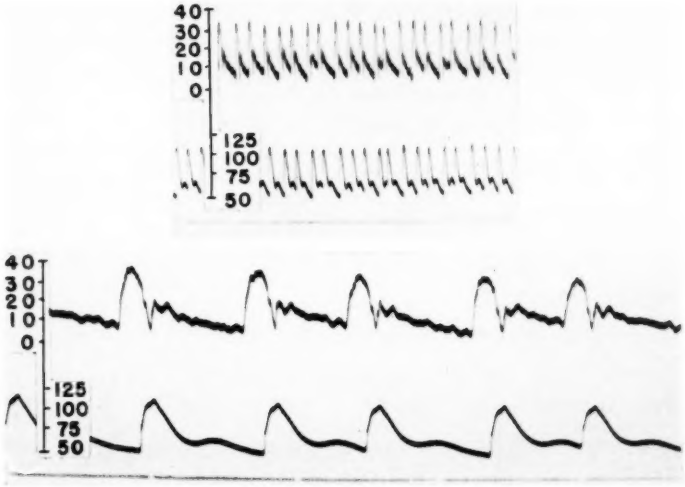

Fig. 2. A typical record at slow speed (upper photograph) and at faster speed (lower photograph) of simultaneously recorded femoral and central pulmonary arterial pressure curves obtained in a trained unanesthetized normotensive dog. Calibration at the left of each figure in millimeters of mercury. In each figure the upper curve is the pulmonary pressure, the lower curve the femoral pressure. Time in seconds.

of the former is the fact that when the unanesthetized animals in our series became uncomfortable both systemic and pulmonary pressure sometimes rose. In favor of the latter is the fact that the lower pulmonary pressure values of Scarff (12) and Johnson, Hamilton, Katz and Weinstein (13) and in our present study on anesthetized animals were obtained with lighter anesthesia.

The effect of renal ischemia on the pulmonary pressure. In both the experiments on anesthetized dogs and those on unanesthetized dogs, the pulmonary arterial pressures did *not* rise when renal ischemia resulted in

a systemic hypertension (cf. tables 1 and 2). This was true from the first day of renal systemic hypertension up to the 99th day. Uremia also had no effect on the pulmonary pressure.

Since the minute volume output of the heart is not significantly altered in renal hypertension (14–18) and the circulating blood volume is unchanged in this state (15, 19, 20, 21) our results indicate that the chemical mediator of renal hypertension apparently does not act upon the pulmonary vessels. Thus, it cannot be a substance like epinephrine which causes a rise in both the systemic and pulmonary arterial pressures (13).

Our results suggest that the chemical mediator of renal hypertension does not operate with equal facility on all the blood vessels. It apparently has no effect on the pulmonary vessels. It is conceivable that it does not act equally on all parts of the systemic circuit. Hence it is possible that in renal hypertension a redistribution of flow in the systemic circuit may occur even though the total minute volume flow of all the parts is unaltered.

Our results suggest that in those cases in which the clinical and necropsy evidence of pulmonary hypertension are seen late in clinical systemic hypertension in the form of pulmonary arteriosclerosis and right ventricular hypertrophy, the changes are probably the indirect result of failure of the left heart and not due to the same type of mechanism responsible for the systemic hypertension.

SUMMARY

The pressure in the central pulmonary artery averages 28/11 and 28/9 mm. Hg respectively in normotensive and hypertensive trained unanesthetized dogs. The range of pressures in the former is $\frac{35 \text{ to } 25}{15 \text{ to } 7}$ mm. Hg and in the latter it is $\frac{37 \text{ to } 21}{18 \text{ to } 5}$ mm. Hg. A method is described for obtaining pulmonary pressures in trained unanesthetized dogs.

Systemic hypertension caused by renal ischemia does not alter the pulmonary pressure. This was demonstrated when readings were made both in anesthetized dogs with chest open and in trained unanesthetized dogs.

The humoral mediator appears to have a selective action on the systemic blood vessels and does not appear to act on the pulmonary vessels.

We are indebted to Dr. M. Friedman for help in the preliminary work, to Dr. H. Sugarman and Mr. S. F. Gaddas for assistance in designing the cannula and to other members of the department for aid in the operations and blood pressure determinations.

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EFFECT OF AGE AND DIET ON ELECTROLYTE CHANGES IN RAT MUSCLE DURING STIMULATION

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It was shown by Fenn and co-workers that stimulation of muscles of rats, cats and frogs results in a loss of potassium in exchange for sodium and a gain of NaCl and water (Fenn et al., 1936, 1938; Fenn, 1937). These changes are largely reversed during recovery. The gain in sodium during activity was confirmed by Malorney and Netter (1936), who used rabbits for their work. Tipton (1938) studied the elctrolyte changes in cat muscles during stimulation and his results were similar to those which Fenn et al. had obtained. He also investigated the effect of injection of KCl, $CaCl_2$ and of cortin on the magnitude of the electrolyte alterations.

In a later publication Fenn (1938) reported the influence of various factors on the loss of potassium from stimulated muscles of cats. It was found that the loss is greater when the muscle pulls against an isometric lever at high initial tension than when the tendon is cut and no external load is imposed. The effects of variations of mechanical movement, blood supply and frequency of stimulation were also investigated.

This report deals with stimulation experiments performed in most cases on young rats of about 5 to 6 weeks of age. The observed changes in potassium and chloride are almost twice as great in the muscles of these animals as they are in those of older rats. The influence of diet, tension, duration of stimulation and poisoning with monoiodoacetic acid has been studied.

METHODS. Rats were anesthetized with urethane, 1.5 gram per kilo of body weight intraperitoneally. The sciatic nerve on one side was exposed and cut in the sciatic notch. After cutting and separating the branch to the hamstring muscles, the peripheral end was drawn into a glass tube and through two silver wire electrodes. The knee on the same side was fixed to a board by means of a stout pin and the Achilles tendon was freed and attached to an isometric bar with ligature thread. The initial tension was automatically set and maintained at any desired value by means of a pulley arrangement described by Fenn (1938). Stimulation was always intermittent, consisting of 0.4 second of tetanus alternating

with 0.4 second of rest. A thyratron stimulator was used to produce the discharges and the frequency in all cases was about 58 shocks per second. Care was taken to keep the stimulus barely maximal. The cathode was attached to the distal electrode.

Immediately after the contraction period the animal was bled to death by cutting the inferior vena cava, after which the gastrocnemius muscle group was removed. No other muscles were ever used. The tissues were freed of fat and nerves and then placed in small weighing bottles. The water content was determined by drying to constant weight at 100°C. For chloride analysis the samples of dried tissue were subjected to the preliminary alkaline digestion which was suggested by Sunderman and Williams (1933), after which the procedure of Van Slyke as modified by Manery et al. (1938) was followed. For the potassium determinations the Shohl and Bennett method as modified by Fenn et al. (1938) was employed. The chloride determinations were not carried out in duplicate because of lack of material. In the case of the potassium analyses, the ashed muscle was extracted with 1N. HCl and duplicate aliquots were taken of the solution.

RESULTS. *Duration of stimulation.* Table 1 shows the effect of duration of stimulation upon the magnitude of the changes in potassium and water.[1] It is evident from the data that the loss of potassium becomes progressively greater as the time of stimulation is increased. However, after 30 minutes there is apparently little further change on continuing the experiment for another half-hour. The gain in water, on the other hand, is at its maximum after only 5 minutes of stimulation. Thereafter there is a tendency for the changes in water to decrease in magnitude. After one hour of stimulation the gain in water is only one-third as much as for the 5 minute period. Certain of the individual results are rather far removed from the average and for this no explanation can be offered. There is no apparent correlation between the variations in tension developed and the variations in potassium changes.

Table 2 shows the results of another series of experiments in which the changes in chloride after varying durations of stimulation were studied. The gain in chloride is almost maximal after 5 minutes of stimulation and shows little variation during the next 25 minutes. After one hour of isometric contraction there is a sharp drop in the amount of chloride gained by the stimulated muscle. The changes in water agree fairly well with those recorded in table 1.

From the increased chloride content the associated gain of extracellular water can be calculated. In the adult rats studied by Fenn it was found

[1] The average potassium content of the resting gastrocnemius of these young rats is 50.5 mM per 100 grams dry weight. The chloride content is 6.2 mM per 100 grams dry weight, and the water content is 77.4 per cent.

that only 42 per cent of the water gained by stimulation is extracellular. However, in these young animals the increase in extracellular water is always equal to more than half of the total gain in water. In fact, beginning with the 15 minute period of stimulation, practically all of the water gained is extracellular. This being the case, it is not surprising that table 2 shows the individual variations in chloride changes being closely

TABLE 1

The loss of potassium and the gain of water by muscles of young rats during stimulation (per 100 grams dry weight)

0		TIME (IN MINUTES) 5		10		15		20		30		60	
K	H_2O	K	H_2O	K	H_2O	K	H_2O	K	H_2O	K	H_2O	K	H_2O
mM	*cc.*	*mM*	*cc.*	*mM*	*cc.*	*mM*	*cc.*	*mM*	*cc.*	*mM*	*cc.*	*mM*	*cc.*
+1.8	+3	2.2	72	5.7	44	10.6	63	12.2	53	9.7	90	11.8	34
−0.4	−8	2.5	80	3.7	115	8.6	52	10.0	42	13.0	75	12.9	4
		2.7	92	5.7	72	8.5	80	9.0	60	14.9	48	11.0	26
0	−4	0.5	54	4.7	68	8.5	63	11.2	82	13.8	36	10.3	31
+0.6	+2	4.6	59	7.0	73	8.5	58	11.3	46	16.4	76	13.3	28
		2.1	75	7.2	54	9.4	57	8.8	60	7.8	40		
				9.5	33			4.7	69	11.5	54		
				3.0	54			8.2	52	11.1	25		
				4.5	58					13.3	30		
				2.3	52					3.2	61		
										10.2	38		
										9.4	40		
										12.3	46		
Av. ΔH_2O.....	−2		72		62		62		58		51		25
Av. ΔK +0.5.....		2.4		5.3		9.0		9.4		11.3		11.9	

Young rats, almost always weighing 75–85 grams, were used. Each pair of figures represents a separate animal. The initial tension in all cases was maintained at 50 grams. During stimulation the muscles developed an additional tension which averaged 100 grams. An isometric lever was used.

The first two columns of tables 1 and 2 refer to experiments in which rats were simply anesthetized for 30 to 240 minutes and then sacrificed. By arbitrarily considering the right leg as the point of reference, it is possible to express the differences between the two sides as + or −.

paralleled by the variations in the gains of water. There is no very evident correlation between the alterations of potassium and of water. Table 1 shows that large losses of potassium are frequently associated with small changes in water content and vice versa.

Fenn (1938) published a few figures on the effect of varying duration of stimulation upon the changes in potassium and water. A more extensive

TABLE 2

The gain of chloride and of water by muscles of young rats during stimulation (per 100 grams dry weight)

TIME (IN MINUTES)													
0		5		10		15		20		30		60	
Cl	H_2O	Cl	H_2O	Cl	H_2O	Cl	H_2O	Cl	H_2O	Cl	H_2O	Cl	H_2O
mM	*cc.*	*mM*	*cc.*	*mM*	*cc.*	*mM*	*cc.*	*mM*	*cc.*	*mM*	*cc.*	*mM*	*cc.*
−0.1	−2	2.9	59	4.8	75	5.9	59	3.5	44	6.0	85	0.09	−12
+0.4	−2	5.2	82	4.0	75	4.6	42	4.6	52	2.7	18	2.3	24
−0.2	−8	2.7	56	4.4	68	2.9	42	4.3	57	5.0	52	2.9	25
−1.2	+4	2.4	61	3.8	72	8.0	87	5.1	53	2.2	16	1.7	14
+0.05	−2	4.0	83	3.1	65			4.5	54	6.2	52	0.5	8
		3.7								5.7	50		
										5.4	43		
										2.1	22		
										2.8	30		
Av. ΔH_2O	−2		68		71		58		52		41		12
Av. ΔCl −0.2		3.5		4.0		5.3		4.4		4.2		1.5	

These figures were obtained with a separate group of rats from those cited in table 1. However, the method of stimulation was kept the same. Consequently, the figures are directly comparable, and the water changes for the two groups should be averaged together. One stimulation experiment has been omitted from the table because it was felt that some gross error in analysis had been made.

TABLE 3

Potassium and water changes during recovery (per 100 grams dry weight)

DURATION OF STIMULATION	TIME ALLOWED FOR RECOVERY	ΔK	ΔH_2O
min.	*min.*	*mM*	*cc.*
10*	0	−5.3	+62
10	10	−3.7	+43
10	25	−3.9	+18
10	45	−1.9	+22
10	45	−3.3	0
10	61	−0.7	0
10	61	−0.7	+3
10	61	−1.0	+4
10	63	−1.4	+2
10	63	+0.1	0
10	65	+0.5	+8

Young rats weighing 75–85 grams were used. The method of stimulation was the same as for tables 1 and 2.

* Average of 10 animals.

study was reported by Tipton (1938). Insofar as the data can be compared our results agree fairly well with these others; there are small points of difference.

Potassium and water changes during recovery. Tipton (1938) and Fenn et al. (1938) have studied the electrolyte changes in muscle during recovery from contraction by various combinations of procedures involving the stimulation of both hind legs. For the data of table 3 separate animals were used and only one side was stimulated. Some of the animals were sacrificed immediately after the period of contraction was over; others were allowed a rest period of varying length before being killed. The results show that an hour's rest suffices to reverse the changes in potassium

TABLE 4

Effect of tension on the changes in water and potassium in young rats (per 100 grams dry weight)

CONTRACTION AGAINST AN ISOMETRIC LEVER		ISOTONIC CONTRACTION AGAINST 50 GRAM LOAD		TENDON CUT (CONTRACTION AGAINST NO LOAD)	
ΔH_2O	ΔK	ΔH_2O	ΔK	ΔH_2O	ΔK
cc.	*mM*	*cc.*	*mM*	*cc.*	*mM*
+53	−12.2	+52	−10.2	+40	−6.5
+42	−10.0	+50	−5.7	+52	−5.6
+60	−9.0	+71	−8.2	+52	−0.9
+82	−11.2	+30	−11.0	+26	−4.5
+46	−11.3			+18	−3.1
+60	−8.8				
+69	−4.7				
+52	−8.2				
Av. +58	−9.4	+51	−8.8	+38	−4.1

Each pair of figures represents a separate experiment. The animals were all stimulated for 20 minutes, using intermittent tetani. The gastrocnemius muscle group was used. The resting tension was maintained at 50 grams, except for the experiments where the tendon was merely cut and no load imposed.

and water which occur as a result of 10 minutes of stimulation. In the experiments of Tipton and Fenn about 3 hours were required to complete recovery, although the changes during stimulation were not very different in magnitude from those reported here. Apparently young rats are able to recover faster than either mature rats or cats.

Effect of tension on potassium loss. Fenn (1938) investigated this problem by stimulating both hind legs of cats. One side was attached to an isometric lever with high initial tension while on the other side the Achilles tendon was cut and the muscle contracted under no load or in some cases under a very light load. The "high tension side" generally contained less potassium, the average difference being 0.9 mM with a probable error of the mean of ± 0.08.

In the present study only one leg was stimulated and separate animals were used to study the effect of varying tension. The results are shown in table 4. It is evident that there is no significant difference in the potassium changes in muscles of rats contracting approximately isometrically and contracting isotonically under a 50 gram load. However, in the group where the tendon was cut and the muscle caused to contract under no external load the average loss of potassium is only 4.1 mM per kilo

TABLE 5

Comparison of the changes in H_2O and K in rats of different age and nutritional history (per 100 grams dry weight)

WEIGHT	POTASSIUM-DEPRIVED RATS				ADULT STOCK RATS			
	Water		Potassium		Water		Potassium	
	Rest.	Stim.	Rest.	Stim.	Rest.	Stim.	Rest.	Stim.
grams	*cc.*	*mM*	*cc.*	*mM*	*cc.*	*mM*	*cc.*	*mM*
64	320	338	28.0	19.6	312	367	49.3	44.2
66	316	405	26.6	23.0	310	367	44.7	40.6
106	308	352	35.2	26.6	308	357	44.5	37.3
80	302	362	28.4	25.2	314*	351	46.3	38.7
108	306	356	35.0	31.4	310*	348	48.0	37.7
99	292	336	24.7	18.4	320	342	50.0	41.5
105	316	352	30.2	22.7	328	350	50.3	42.7
56	306	340	27.7	22.5	312*	344	46.5	38.2
Av. change...	+47		−5.8		+39		−7.3	
Av. in adult rats; Fenn and Cobb (1936).....					+49		−6.1	
Av. in young rats; see table 1.....					+51		−11.3	

The period of stimulation was 30 minutes. The gastrocnemius muscle group pulled against an isometric lever and the initial, resting tension for the potassium-deprived rats was set at 50 grams. The muscles of the mature rats, except for the first two in the table, were also under a fixed initial tension, which was either 150 or 200 grams. Muscles of the animals studied by Fenn and Cobb were not under any fixed initial tension.

* These rats received the same diet as the potassium-deprived animals except that it was supplemented with 0.58 per cent of potassium. All other rats mentioned in this paper received a stock diet of calf meal or Purina Dog Chow.

dry weight, compared with 9.4 mM for the "high tension" animals. The difference between these two means is 5.3 and the standard deviation of the mean difference is 0.49. The variation is therefore significant. It should be noted that the magnitude of the change in water content also tends to decrease with decreasing tension.

Effect of age and diet. From table 5 it is evident that young rats incur a much greater loss of potassium from stimulated muscles than do mature

rats. Of course, the total tension developed is quite different in the two groups of animals and the tension per unit area of muscle cross section is probably not the same. For this reason comparisons may not be justified. Nevertheless, the fact remains that by no adjustment of initial tension or by any other means has it been possible to obtain potassium changes in the muscles of older rats which equal those observed in young animals.

In table 5 are also contained the results of stimulation experiments carried out on potassium-low rats. The preparation of diets and the electrolyte pattern of certain tissues of these animals has already been described (Heppel, 1939). In these rats the potassium content of muscle drops to almost one-half of the normal value and there is a remarkable gain of sodium, most of the sodium occurring inside muscle cells. It is seen from table 5 that the altered electrolyte picture in these muscles does not greatly affect the results obtained in stimulation experiments. The loss of potassium is less than is the case for stock rats of the same approximate weight; however, the tension developed is also less and this may partially explain the differences. Moreover, the potassium-deficient animals, when sacrificed, were about 4 weeks older than stock animals of the same weight because there was no gain of weight during the depletion period. As already mentioned the potassium changes are of lesser magnitude in older rats. It is interesting to note that the potassium content of the stimulated muscles of these animals falls to values as low as 18.4 mM per 100 grams dry weight. This is only 36 per cent of the normal resting potassium level.

Bruman and Finkelstein (1936) maintained rats of 150 to 200 grams for several weeks on a diet of milk and bread to which was added each day a solution containing 0.2 gram of potassium. By this procedure they were able to double the potassium content of resting muscle. Since it would be interesting to study the behavior of muscles with such a high content of potassium, we raised 4 male rats, of 40 grams initial weight, on a diet containing 2.0 per cent of this element. One of the animals was sacrificed when it weighed 80 grams; the others remained on the diet until from 31 to 50 days after they had achieved a weight of 150 grams. Food intake measurements showed that the rats ate 10 grams or more per day; consequently the daily potassium intake was at least 0.2 gram. The resting potassium content of muscle was normal in all 4 rats and the loss on stimulation, determined on 3 rats, was not unusual. It is unfair to controvert the data of Bruman and Finkelstein when so few animals were used, but at least it becomes a little questionable whether the potassium content of muscle can really be doubled by increasing the dietary intake.

Effect of injection of iodoacetic acid. In recent years a fair amount of evidence has accumulated showing a relation between potassium and carbohydrate metabolism. Thus, the injection of subtoxic amounts of potassium salts into rats was found to result in a marked rise in blood sugar and

decreases in liver, muscle and cardiac glycogen (Silvette et al., 1938). Conversely, the continuous intravenous injection of glucose into normal dogs produced a fall in serum potassium and in phosphate (Flock et al., 1938).

Monoiodoacetic acid in toxic doses causes a hyperglycemia and arrests lactic acid formation in muscle and other tissues (Irving, 1933; Nahum and Hoff, 1934). A few experiments were tried in which this substance was injected intraperitoneally and the effect on the resting potassium level and the loss of potassium on stimulation determined.

In the first group of 6 experiments, adult rats were anesthetized and the left gastrocnemius muscle group was tied off and sampled. Then 130 to 200 mgm. of monoiodoacetic acid were injected, which caused death in from 15 to 90 minutes followed by immediate rigor. The right gastrocnemius was then removed. There was no significant difference in the content of potassium or of water before and after poisoning. In another group of four experiments the muscles were stimulated as already described. The acid was injected just after contractions began and it caused the tension developed to diminish rapidly. The average loss of potassium was 4 mM per 100 grams dry weight, compared with 7.3 for normal adult rats. A smaller fall was to be expected in view of the low tension developed. The experiments were unsatisfactory in that the potassium changes may have occurred before the acid had completely taken effect. It would have been better to have followed the content of potassium and lactic acid in blood before and after the poisoning.

SUMMARY

1. The loss of potassium on stimulation of rat muscles is progressively greater as the duration of contraction is increased. After 30 minutes there is no further change, at least for another half-hour.

2. The changes in chloride and water are almost maximal after 5 minutes of stimulation; after 1 hour the changes are very much reduced in magnitude.

3. The individual variations in the water changes follow closely those of chloride, but there is no correlation with the variations in the potassium changes. The potassium loss and the chloride gain apparently bear a different relationship to the contraction process.

4. Previous work relating to the changes during recovery is confirmed.

5. Young rats of about 6 weeks of age are able to lose more potassium from their muscles on stimulation than is the case for adult rats.

6. Feeding diets low in potassium and poisoning with monoiodoacetic acid have little or no effect on the potassium changes during stimulation. Previous work on the influence of tension is confirmed.

Acknowledgment. I am very grateful to Dr. W. O. Fenn for help rendered throughout this investigation and for many useful suggestions.

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THE DIFFUSION OF RADIOACTIVE SODIUM INTO THE MUSCLES OF POTASSIUM-DEPRIVED RATS

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The muscle fiber seems to be normally impermeable to anions and to the larger cations, such as sodium, lithium and calcium. It is permeable to potassium, cesium, rubidium, ammonium and hydrogen ions. It is believed on the basis of mobility measurements that lithium and sodium cannot penetrate the membrane of muscle cells because of their degree of hydration, which increases the effective ionic diameter (Mond and Amson, 1928).

The extracellular phase of muscle is thought to approximate a serum ultrafiltrate in composition and this phase is held to contain all of the chlorine and practically all of the sodium. In a number of species of animals, however, a certain amount of "excess sodium" remains after due allowance has been made for that portion which is in the extracellular phase (Fenn, Cobb and Marsh, 1934; Mond and Netter, 1932; Harrison et al., 1936; Hastings and Eichelberger, 1937). It is something of a problem to locate this sodium which must be elsewhere than with chloride in the interstitial fluid. Mond and Netter consider that it is combined in some way in the surface of the muscle fibers. Fenn (1936) has expressed the opinion that it might possibly be inside the fibers along with potassium, but unable to penetrate the membrane.

In the muscles of potassium-deprived rats the content of potassium is greatly decreased while sodium is increased over two-fold in amount (Heppel, 1939). Most of the sodium in such muscles is intracellular; that is, it cannot be accounted for in the same phase which contains the chloride. It is a matter of some interest to determine whether this relatively very large amount of "excess sodium" readily exchanges with the sodium in the plasma or whether it is irreversibly held within the muscle cells. In the present investigation this question has been studied by using radioactive sodium as an indicator.[1]

METHODS. The preparation of potassium-low diets and the analytical

[1] Cohn and Cohn (1939) have studied the permeation of radioactive sodium into red corpuscles of the dog.

methods which were used have already been described (Heppel, 1939). Young albino rats weighing 90 to 100 grams were employed for the experiments. After being maintained on the deficient diet for a suitable period of time the animals received an intraperitoneal injection of isotonic, radioactive sodium chloride solution. (A stomach tube was used for rats 7 and 1a; cf. table 1.) After varying intervals of time, they were killed by decapitation and the blood was collected in centrifuge tubes. Serum was removed after centrifugation and samples taken for determination of sodium and chloride. The hind-leg muscles were quickly dissected off

TABLE 1

The penetration of Na^{24} into the muscles of potassium-deprived rats

(1)	(2)	(3)	(4)	(5)		(6)	(7)		(8)	(9)		(10)
RAT NUMBER	WEIGHT	DAYS ON DIET	TIME*	Na^{24}, COUNTS/GM./MIN.		$\frac{A}{B} \times 100$	SODIUM, mM/KILO FRESH TISSUE		$\frac{C}{D} \times 100$	CHLORIDE, mM/KILO FRESH TISSUE		$\frac{E}{F} \times 100$
				(A) Muscle	(B) Serum		(C) Muscle	(D) Serum		(E) Muscle	(F) Serum	
	grams		*min.*									
5	117	40	5	640	4700	14	53.0	135†	39.2	10.2	87	11.7
4	101	36	10	770	4500	17	32.4	138	23.5	11.6	93	12.5
8	90	34	10	1000	6600	15	35.4	132	26.8			
1	102	40	20	960	4200	23	51.0	140	36.4	8.8	91.9	9.6
3	117	37	31	350	1390	25	48.1	140	34.4	9.5	92.5	10.3
9	80	37	60	430	1400	31	43.8	134	32.7	11.9	98	12.1
14	98	44	60	140	430	33	46.0	131	35.0			
10	108	34	182	1500	5300	28	39.8	128	31.1			
7	110	44	187	90	240	38	56.0	136	41.2			
1a	101	42	215	730	2300	32	43.2	143	30.2			
2	99	34	260	1320	4000	33	44.7	137	32.6			

* This is the time between giving the radioactive solution and sacrificing the animal.

† The sample was lost and the average value for serum sodium has been substituted. The experiment with rat 1 was marred in that a large part of the injection was only subcutaneous.

and freed of gross masses of fat and connective tissue. They were weighed in covered platinum crucibles after which they were treated with sulfuric acid and ashed in a muffle furnace. In some cases an aliquot of the muscle tissue was transferred to a large centrifuge tube and used for chloride analysis. It was not possible to carry out the determinations in duplicate because of the limited amount of sample.

Radioactivity was determined on extracts of the ashed tissue by the method developed by W. J. Bale et al. (1939) in the Department of Radiology using a Geiger-Müller counter. The average difference between duplicate counts was 4.3 per cent; 10.0 per cent was considered the outer limit for acceptable values.

RESULTS. Table 1 gives the data from 11 experiments in which rats were sacrificed at varying intervals of time after receiving a solution containing radioactive sodium.[2] The ratio of Na^{24} per unit weight of muscle to Na^{24} per unit weight of serum has been calculated for each rat, and the results are given in column 6. The ratios of total sodium in muscle compared to serum have also been calculated; the figures are shown in column 8. When the radioactive isotope has effected complete penetration into the muscles from the blood stream, then the two ratios will be equal. It is evident from the table that within 60 minutes the radioactive isotope was distributed between plasma and muscle in the same proportion as the total sodium. Since up to two-thirds of the muscle sodium is intracellular in potassium-deficient rats, the cell membranes are apparently permeable to sodium.

The experiments involving shorter intervals of time showed only partial penetration of Na^{24} into the intracellular phase. However, complete interchange with the extracellular fraction of the muscle sodium occurred even in the space of 5 minutes. Column 6 contains the ratios of muscle chloride to serum chloride, which represent a sufficiently accurate measure of the extracellular phase for present purposes. In previous work the average magnitude of this phase was found to be 13.4 per cent.

Manery and Bale (1939) carried out similar experiments using normal rats, in which practically all of the sodium of muscle is extracellular. Permeation of Na^{24} into muscle was complete in eight minutes.

The suggestion that the "excess sodium" might exist inside the fibers along with potassium but be unable to penetrate the muscle membrane is apparently not valid as far as potassium-deficient rats are concerned. In normal animals the problem is somewhat harder to study because the "excess sodium" amounts to only 10 per cent of the total, and the error involved in using the Geiger-Müller counter is of this order. The theory of Mond and Netter (1932) also appears unsatisfactory. In potassium-deprived rats the concentration of sodium rivals that of potassium and it seems unlikely that two-thirds of it should be combined on the surface of the muscle fibers. Such a situation would make for a serious deficiency in osmotically active cations in the intracellular phase, unless compensating changes took place involving other constituents of the muscle cell.

The hypothesis that the muscle membrane of these animals is permeable to sodium also offers difficulties. It becomes necessary to explain how potassium is maintained within the cell against a concentration gradient of about 40:1 and how sodium is maintained outside of the cell against a

[2] The figures are expressed as counts per gram per minute. The strength of the solution used was of the order of 200,000 counts/cc./min. and 3 cc. per 100 grams body weight were injected. There was very wide variation in the amount of radioactive sodium introduced because the same solution was used for experiments on successive days if decay was not too great.

concentration gradient of about 2.5:1, if the membrane allows the interchange of both of these elements.

The potassium content of spleen, kidney and heart. In a previous report it was shown that liver tissue of potassium-deprived rats suffered no change in its content of potassium, while in the case of skeletal muscle and serum there was a drop of about 50 per cent. Table 2 contains data for three other tissues. The diet contained less than 0.01 per cent of this element.

The deficiency resulted in a small decrease in the potassium content of spleen. There was a similar reduction in the case of kidney. The indi-

TABLE 2

The potassium and water content of spleen, kidney and heart in potassium-deprived rats (per kilo fresh tissue)

	WEIGHT OF RAT	SPLEEN		KIDNEY		HEART	
		K	H_2O	K	H_2O	K	H_2O
	grams	*mM*	*grams*	*mM*	*grams*	*mM*	*grams*
Potassium deprived rats	94	123.4	776	60.4	785	72.7	780
	82	103.1	770	71.4	772	80.5	770
	90	122.0	775	57.4	805	71.1	778
	92	118.2	776	51.2	828	69.2	785
	116	113.6	768	63.7	797	68.2	790
Average		116.1	773	60.8	797	72.3	781
Control rats	207	137.2	780	77.9	772	81.6	782
	144	130.0	785	79.5	770	84.8	775
	178	130.5	779	80.4	771	87.4	770
Adult stock rats*		113.1	766	71.1	762	84.0	780

The rats were killed by decapitation. The tissues were trimmed and blotted, and for heart everything above the A-V septum was cut away. The rest of the procedure has already been described (Heppel, 1939).

* Data from Manery and Hastings (1939).

vidual variations in the potassium content of kidney were large, and they bore an inverse relationship to the variations in water content. The ventricles of the heart also showed a drop in the level of potassium, but the change was far less than that observed for striated skeletal muscle.

Pathological changes.[3] A careful gross examination of the organs of 12 potassium-deficient rats was carried out, and paraffine sections were prepared of certain tissues of 5 of these animals. The microscopic sections were stained by the ordinary H. and E. method.

[3] Dr. S. Madden of the Pathology Department was kind enough to render assistance with the examinations.

A striking enlargement of the kidneys was consistently found. The average weight of this organ was 1 per cent of the total body weight for the potassium-deprived rats, and only 0.4 per cent for the control animals. Grossly, the organ was paler than usual, but not otherwise remarkable. Histologically, marked dilatation of the collecting tubules was observed, with pycnosis of the nuclei of the epithelial cells and considerable sloughing of the epithelium. The convoluted tubules showed a little dilatation. There was considerable congestion of the renal blood vessels. No changes were evident in the glomeruli.

This confirms the findings of Schrader, Prickett and Salmon (1937) who investigated the pathological alterations which occurred because of a deficiency of potassium in the diet. Our results, however, differ from theirs on the following points: ascites and hydrothorax were never noted. The intestinal tract was either not enlarged at all or only slightly enlarged. It was usually not markedly atonic. Intussusceptions and areas of annular thickening in the ileum were never noted. The microscopic sections were poor and it was impossible to study the changes in the villi. The suprarenal glands showed neither congestion nor hemorrhage on gross examination. Microscopically, there was some congestion in the vessels of the medulla.

We were able to confirm the work of the above-mentioned workers on the following points: the mesenteric lymph nodes were enlarged and translucent in some of the animals. Microscopically, they showed edema and the presence of numbers of mono-nuclear phagocytes. The pancreas was dissociated into its constituent lobules to a greater extent than in the control animals. We were occasionally able to find opaque areas at the apex of the heart. Sections through these areas showed erosion of the myocardium and replacement with scar tissue. Microscopically, there was some indication of foaminess of hepatic cells, but grossly the liver was not unusual.

Heppel and Schmidt (1938) reported that potassium-deprived rats suffered from progressive abdominal distension and at autopsy showed a very flaccid and dilated gastrointestinal tract. In the present investigation the same diet was used but these changes were either absent or slight in degree. It is likely that the particular batches of liver extract and rice bran extract which were used provided a richer source of water soluble vitamins than was available in the previous work. If it were possible to feed super-abundant amounts of all of the components of the vitamin B complex without introducing large amounts of potassium at the same time it might be possible to keep young rats on a potassium-deficient diet in much better condition than is possible at present. The animals studied by Schrader, Prickett and Salmon may have had insufficient quantities of certain of the water soluble vitamins, for their control rats gained less than 1 gram a day.

SUMMARY

1. The penetration of sodium into the muscles of potassium-deprived rats has been studied by means of the artificially prepared radioactive isotope of this element.

2. Both the extracellular and intracellular fractions of the muscle sodium exchanged readily with the sodium in the plasma. The membranes of the muscle cells were apparently permeable to sodium.

3. In potassium-deprived rats a decrease was found in the content of this element in spleen, kidney and heart, but the changes were small compared to the loss incurred by skeletal muscle.

4. Previous work on the pathological findings in potassium deficiency has been partly confirmed. There were certain points of difference.

Acknowledgment. I am grateful to Dr. W. O. Fenn for advice and assistance during the course of this work. To Miss J. F. Manery I am indebted for several useful suggestions, and for help in carrying out several of the earlier experiments. Miss Eugenia Sheridan carried out the potassium analyses reported here.

The supplies of radioactive sodium were very kindly made available by Dr. L. A. DuBridge and Dr. S. N. Van Voorhis of the University physics department operating under a grant from the Rockefeller Foundation. On one occasion Dr. Robert F. Bacher of Cornell University was good enough to fill an emergency need for the material. I am indebted to Dr. W. F. Bale for supervising the operation of the Geiger-Müller counter.

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THE METABOLISM OF RABBIT BONE MARROW IN SERUM[1]

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This paper presents a comparison between the metabolism of slices of rabbit bone marrow suspended in serum and in Ringer solutions. Orr and Stickland in 1938 (1) studied the metabolism of suspensions of rabbit bone marrow in Ringer solutions and concluded that the marrow has a type of metabolism similar to "that of tumour tissue, retina and kidney medulla" in that the ratios Q_{O_2}:$Q_G^{O_2}$:$Q_G^{N_2}$ they found were roughly 1:1:2. The absolute values, however, were smaller in the case of marrow. Experiments conducted by the writer in the laboratory of Dr. Ephraim Shorr several years ago suggested that there might be distinct differences in the metabolism of rabbit bone marrow depending upon whether serum or Ringer solution is used as the suspension medium. The present paper is an extension and confirmation of these findings.

Respiration. In the usual methods of measuring oxygen consumption in serum, with the exception of the Warburg 2-vessel technique, a single figure is obtained for the amount of oxygen consumed during the entire course of the experiment. In converting this figure into a *rate* of oxygen consumption (Q_{O_2}), one is forced to assume that the oxygen has been consumed at a constant rate throughout the experiment. This assumption may or may not be justified and it was accordingly desirable to develop a convenient method for obtaining consecutive readings of the rate of oxygen consumption in serum with a single sample of tissue. If one attempts to use unaltered serum in ordinary Warburg respirometers, all of the CO_2 (including bicarbonate-CO_2) is absorbed by the KOH present and the pH is shifted out of the physiological range. The dependence of serum pH on CO_2 tension may be eliminated by partial decomposition of the bicarbonate with acid, followed by evacuation of the serum and buffering as described below. Such serum is hereafter referred to as "neutralized" serum although it is clear that what is meant is a serum which remains physiologically neutral when equilibrated with a CO_2-free atmosphere.

[1] The data of this paper were presented at the Toronto meeting of the American Physiological Society, May, 1939.

[2] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

It is not, however, strictly bicarbonate-free serum as described by Canzanelli and Rapport (2).

The serum is "neutralized" by adding 0.1 N HCl to such a pH (approximately 6.6) that upon removal of the CO_2 by evacuation the pH is restored to about 7.4. It is then buffered at this level by adding one part of M/15 pH 7.4 phosphate buffer to 10 parts of serum. Such serum may be used in the ordinary Warburg vessels (as has also been shown by MacLeod and Rhoads, 3) and, as the data in table 1 show, is entirely comparable to unaltered serum with respect to its effect on bone marrow respiration. The measurements using untreated serum were made with a Dickens-Greville manometer employing small (13–14 cc.) Dixon-Keilin vessels. The sensitivity of this instrument is about 15 times that of the ordinary Dixon-Keilin apparatus, and the vessels, in the experience of the author, are more

TABLE 1

Comparison of the rate of respiration of normal rabbit bone marrow in serum and Ringer solution

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
EXPERIMENT NUMBER	Q_{O_2} IN FIRST HOUR IN				INCREASE IN Q_{O_2} IN SERUM	Q_{O_2} IN 5TH HOUR COMPARED WITH Q_{O_2} IN FIRST HOUR	
	Neutralized rabbit serum	Neutralized horse serum	Unaltered rabbit serum	Ringer-phosphate glucose		in serum	in Ringer
					per cent	*per cent*	*per cent*
55	−5.7	−5.9	−5.8	−3.6	60	95	79
56	−6.2	−6.2	−6.5	−4.1	59	94	73
58	−6.0	−6.1	−5.7	−3.1	94	98	83
72	−6.2	−6.5	−6.5	−3.9	67	Only run 2 hours	
90	−6.3	−5.7	−6.1	−3.5	75	98	84
Average.....	−6.1	−6.1	−6.3	−3.7	70	96	80

dependable than those of the Dickens-Greville type. In this connection one should mention, however, that the recently developed Summerson Combination Manometer (4) combines both these features in a much more rugged and easily-operated instrument. The marrow slices were prepared by the method already described (5) but no attempt has been made, as in the earlier publication, to relate the metabolism of the marrow to its cellular constituency. The oxygen consumption and glycolysis figures in this paper are reported in $mm.^3$ per mgm. fat-free dry weight of tissue per hour. The fat-free dry weights were calculated from nitrogen determinations made on the marrow samples using the fact established by Orr and Stickland that nitrogen constitutes on the average 14.6 per cent of the fat-free dry weight of rabbit bone marrow. All experiments were conducted at 37.5°C. The Ringer solutions were prepared as follows:

Ringer solution: 0.154 M NaCl 96 parts, 0.154 M KCl 2 parts, 0.111 M $CaCl_2$ 2 parts.

Ringer-phosphate-glucose solution: 9 parts of the above Ringer solution plus 1 part of M/15 pH 7.4 phosphate buffer plus glucose to make a concentration of 200 mgm. per 100 cc.

Ringer-bicarbonate-glucose: 8 parts Ringer solution plus 2 parts 1.05 per cent $NaHCO_3$ solution plus glucose to 200 mgm. per 100 cc.

Further reference to table 1 shows that the rate of respiration is the same in neutralized rabbit or horse serum in the Warburg manometers as it is in unaltered serum in the differential manometer (column 4). The rabbit sera are from the same animals as the marrows. The comparison

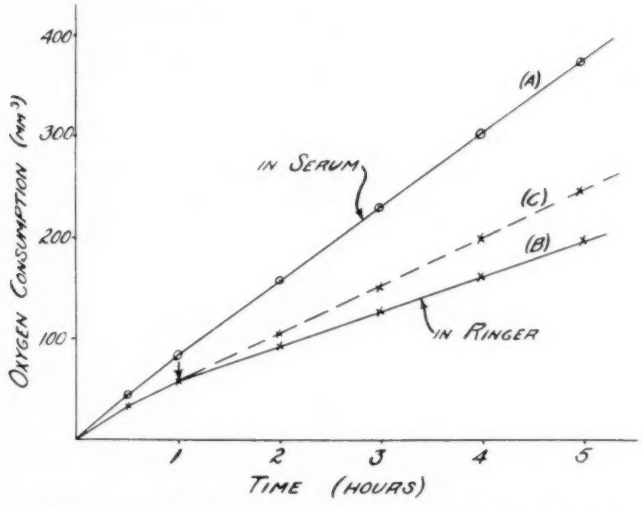

Fig. 1. Respiration of bone marrow in serum vs. Ringer's solution. Curve *C* shows the effect of adding 0.5 cc. of serum to 2 cc. of Ringer-phosphate-glucose medium at the end of the first hour (arrow).

between respiration in serum and Ringer-phosphate-glucose solution, however, shows entirely different results. In all the serum experiments the Q_{O_2} averages 70 per cent higher than corresponding readings in Ringer-phosphate-glucose medium. In each case, controls were run which demonstrated that the oxygen consumption of the serum itself was negligible. Furthermore, the rate of respiration in neutralized serum (columns 7 and 8) is maintained for 5 hours at about 96 per cent of its rate during the first hour, whereas in Ringer solution the rate falls off so that during the fifth hour it is only about 80 per cent of the initial rate. Figure 1 shows the time-course of respiration in neutralized serum versus Ringer-phosphate-glucose medium (one of 4 entirely comparable experiments), and the effect of adding 0.5 cc. of serum to the 2 cc. of Ringer solution is also

indicated. The linear time-course of at least 5 hours' duration, induced by the use of serum, is uncommon in tissue metabolism experiments in the experience of the author.

Anaerobic glycolysis. The comparison of anaerobic glycolysis of normal rabbit bone marrow in serum and in Ringer-bicarbonate solution is presented in table 2. In obtaining these data the following procedures were used. In all instances, after a preliminary flushing of the vessels with oxygen-free nitrogen, carbon monoxide was run through the vessels to remove the last traces of oxygen which might otherwise have remained in combination with the hemoglobin in the marrow. The excess carbon mon-

TABLE 2

Comparison of anaerobic glycolysis in serum and Ringer-bicarbonate-glucose solution

(1)	(2)	(3)	(4)	(5)	(6)	(7)
EXPERIMENT NUMBER	$Q_G^{N_2-CO}$ IN RINGER-BICARB. SOLUTION	$Q_G^{N_2-CO}$ IN HORSE SERUM	INCREASE IN RATE IN HORSE SERUM	$Q_G^{N_2-CO}$ IN RABBIT SERUM	INCREASE IN RATE IN RABBIT SERUM	LACTIC ACID RECOVERED (CHEM.) IN SERUM
			per cent		*per cent*	*per cent*
60	8.2	11.2	37			
61	6.6	9.7	46			85
62	11.1	13.8	24			86
63	7.0			10.6	51	98
64	11.0			11.2	2	103
65	8.5	13.2	55			
66	8.7	14.2	63			
67	8.0	10.6	32	12.4	55	
69	8.2	13.4	63	14.9	82	91*
71	8.5	12.6	48	14.9	75	77*
Average	8.6	12.3	43.5	12.8	66†	90

* In rabbit serum.

† Not including experiment 64.

oxide was then removed with 95 per cent N_2-5 per cent CO_2 mixture, and it was found desirable to do this while the vessels were being shaken in the water bath. The retention of CO_2 was determined for each serum by dumping standardized acid into the serum and Ringer-bicarbonate solution and comparing the amounts of CO_2 liberated from the two media. The manometer readings in the experimental vessels were then corrected for the percentage of CO_2 retained in the serum. The validity of this procedure was tested by comparing the glycolysis figures so obtained with chemical determinations of the lactic acid production by the same marrow slices using the method of Friedmann, Contonio and Shaffer.

Table 2 shows that in 9 out of 10 instances the rate of anaerobic glycoly-

sis is greater in serum than in Ringer-bicarbonate-glucose medium and the last 3 experiments show that it is even greater in rabbit serum than in inactivated horse serum. The increment in rabbit serum (or, more properly speaking, the decrement in the Ringer solution) is variable but, as in the case of respiration, is of the order of 70 per cent.

These results are confirmed by the lactic acid analyses. One sees (column 7) that the amount of lactic acid calculated from the corrected manometer readings, on the average is 90 per cent recoverable by the chemical analysis, so that if acids other than lactic acid are produced by the marrow under anaerobic conditions, they do not constitute more than 10 per cent of the total acid production. Actually, this discrepancy between the manometric and chemical determinations of lactic acid is partly to be explained by the fact that pure lactate added to the serum was only about 95–97 per cent recovered by the chemical procedures used.

TABLE 3

Comparison of the rate of aerobic glycolysis in serum and Ringer-bicarbonate-glucose solution

(1)	(2)	(3)	(4)	(5)
EXPERIMENT NUMBER	$Q_G^{O_2}$ IN RINGER SOLUTION (MANOMETRIC)	$Q_G^{O_2}$ IN RINGER SOLUTION (CHEMICAL)	$Q_G^{O_2}$ IN RABBIT SERUM (CHEMICAL)	R.Q. IN RABBIT SERUM
73	2.0		1.5	0.880
77	2.0	1.9	3.2	0.875
78	2.0	1.9	1.9	0.870
79	2.3	2.3	2.4	0.880
80	2.1	2.1	2.9	0.935
Average.....	2.1	2.1	2.4	0.886

Aerobic glycolysis. The comparison of aerobic glycolysis in serum with that in Ringer-bicarbonate-glucose medium is a much more difficult procedure than in the case of anaerobic glycolysis. In fact, at the time this work was done, there was no simple manometric technique for measuring aerobic lactic acid production in serum with proved accuracy. (See, however, (6) in which a new method is described which is apparently applicable to this problem.) Under the circumstances, therefore, it appeared desirable to inquire whether differences in the rates of aerobic glycolysis in the two media could be demonstrated by chemical means. The results are shown in table 3 in which the chemical analyses have been converted into $Q_G^{O_2}$ values for comparison with the manometric determinations. In addition, in order to calculate these figures from the manometric data, the R. Q. of the tissue must be known. It was found most convenient to make these measurements in serum in the differential manometer; the results

are given in the last column. It is implied that the R. Q.'s are essentially the same in the two media, and the close agreement between the manometric and chemical estimations of $Q_G^{O_2}$ in Ringer solution suggests the validity of this assumption.

The table also shows that there are large variations in the chemical estimation of lactic acid production in serum, probably due to the fact that one is attempting to measure rather small increments in a relatively large lactic acid content of the serum. With such variations in the individual experiments, the findings that the chemical estimation in serum averages 14 per cent higher than that in Ringer solution is of doubtful significance. One may interpret these findings as follows: 1, the aerobic glycolysis of rabbit bone marrow is in any case small, and 2, such differences as may exist between the rates in the two media are probably within the limits of experimental error.

Discussion. The average figures for the various phases of metabolism in the above experiments are very close to those of seven other manometric experiments without chemical determinations which have yielded the following averages: $Q_{O_2} = -6.2$, $Q_G^{O_2} = 2.1$, $Q_G^{N_2-CO} = 12.8$. These figures show that the metabolism of normal rabbit bone marrow in serum is quite different from that deduced from the measurements made in similar Ringer solutions by Orr and Stickland (1). The comparison of the two sets of data is presented in table 4. One sees that whereas we are in essential agreement as regards the findings in Ringer solutions, where the aerobic glycolysis is nearly as high as the respiration, in serum the rate of respiration is about $2\frac{1}{2}$ times that of the aerobic glycolysis. In serum, therefore, the marrow does not exhibit a metabolism characteristic of tumour tissue, but closely approximates that of other adult normal tissues, in particular lymphocytic tissue such as lymph gland and tonsil (cf. Burk, 7, table 1). It is true that the rate of aerobic glycolysis is rather higher than with many normal tissues, but it has been shown by MacLeod and Rhoads (3) that leucocytes (from rabbit exudates) have a high rate of aerobic glycolysis even in serum and it is likely that aerobic glycolysis in marrow is due in large part to its granulocyte content. In any case, it is clear that changing the medium from Ringer solution to the more physio-

TABLE 4

Summary

MEDIUM	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2-CO}$	MEYERHOF QUOTIENT	SOURCE
Ringer	−3.9	+3.8	+7.1	2.5	Orr and Stickland, 1938
Ringer	−3.7	+2.1	+8.6	5.3	This paper
Serum	−6.3	+2.4	+12.8	5.0	This paper

logical serum results in a still more normal type of metabolism. It is also of interest to note that the Meyerhof oxidative quotients found in the present study, in either medium, are somewhat higher than those calculated from Orr and Stickland's data.

Fugita (8) studied the effect of Ringer solution on the metabolism of suspensions of rat bone marrow and found that in this medium the respiration progressively decreased to zero, with a corresponding increase in the rate of aerobic glycolysis to nearly the anaerobic level. The writer's experience is in agreement with that of Orr and Stickland in never finding this effect in the case of rabbit bone marrow.

Considerably higher rates of respiration in serum, as compared with Ringer solution, have been observed in various other tissues, namely, nerve, liver and kidney (9) (10) (11) (2), and possibly testis (2) (9), but the opposite has been observed in the case of brain (2). Dickens and Simer did not note these differences in their original report (12). Moreover, there is no unanimity of opinion as to what substance or substances in serum are responsible for these effects. This problem is being investigated.

SUMMARY AND CONCLUSIONS

1. The use and advantages of "neutralized" serum in bone marrow respiration studies are described. The Q_{O_2} values obtained in ordinary Warburg manometers with this medium are the same as those obtained in differential manometers using unaltered serum and marrow taken from the same animal.

2. The rate of respiration of slices of normal rabbit bone marrow is about 70 per cent higher in serum than in Ringer-phosphate-glucose medium and is maintained at a constant rate for at least 5 hours.

3. The rate of anaerobic glycolysis is also about 70 per cent higher in serum than in Ringer-bicarbonate-glucose medium; this finding was checked chemically.

4. Chemical studies of aerobic glycolysis in serum and in Ringer-bicarbonate-glucose solution suggest that differences in the $Q_G^{O_2}$ values in these two media are probably within the limits of experimental error.

5. The metabolism of normal rabbit bone marrow in serum is essentially comparable to that of other adult normal tissues, in particular lymphocytic tissue. A "tumour" type of metabolism in Ringer solution, as described by Orr and Stickland, is not found when the measurements are made in serum.

6. The mechanism of the serum effects is under investigation.

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PREGANGLIONIC NERVE REGENERATION IN COMPLETELY SYMPATHECTOMIZED CATS

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The physiology of the completely sympathectomized animal was first studied by Cannon and his co-workers (1929). Animals deprived of sympathico-adrenal influences have been followed by various investigators over a period of months and even years. It is therefore of obvious importance to know whether sympathetic regrowth may occur to complicate such long-term studies. Physiological data on this question are relatively sparse.

Langley (1897) sectioned the cervical sympathetic trunk and found almost normal sympathetic activity 24 days afterwards. He believed that functional regeneration began to appear on about the 12th day. Bacq and Dworkin (1930) obtained evidence of sympathetic regrowth in 2 cats about a year subsequent to complete sympathectomy. Maes and Simeone (1938) crushed the splanchnic nerves and reported return of medulliadrenal function after 50 days. Hodes (1939) indicated the possibility of regrowth in 2 cats deprived of the sympathico-adrenal system. Hinsey, Phillips and Hare (1939) sectioned thoracic ventral roots and noted preganglionic regeneration at various times thereafter. They also observed a re-establishment of connection between preganglionic fibers and cells in the cervical ganglia after thoracic ganglionectomy and sympathectomy.

Most previous workers have studied regeneration after section of nerves or partial sympathectomy. The following data were collected with the view of adding further observations to the still-incomplete reports on regeneration in the totally sympathectomized animal.

METHODS. Cats were sympathectomized in three stages according to the technique of Cannon, Newton, Bright, Menkin and Moore (1929). Generally, a large portion of the splanchnic nerves was removed with the chains, and in some animals the celiac ganglia were extirpated; except for one animal in which unilateral superior cervical ganglionectomy was performed, the sympathetic trunks in the neck were left intact. The cats were sacrificed 54 to 236 days after the final operation.

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After anesthetization with urethane (1.25 grams per kgm. intravenously) the animals were placed on a heating pad and a tracheal cannula was inserted. Both vagi were cut in the neck. The sciatic, median, or saphenous nerve on one side was exposed and prepared for central stimulation. Both cervical sympathetic trunks were dissected and the cut cephalic end or the intact nerve was stimulated.

TABLE 1

Observations after last stage of sympathectomy. The pupillary changes resulted from stimulation of the cervical sympathetic trunks; the changes in heart rate resulted from stimulation of an afferent nerve

CAT NUMBER	DAYS AFTER SYMPATHECTOMY		ACTION OF PUPIL AND NICTITATING MEMBRANE		HEART RATE PER MINUTE		REMARKS
	First stage	Last stage	Left*	Right	Before	Highest rate after stimulation	
1	163	54	+	0	218 210	218 210	Celiac ganglia removed. At autopsy fragments of 2 thoracic ganglia were found
2	173	71	+	Slight reaction with strong currents	218	220	
3	184	100	+	+	223	225	
4	224	124	+	+	206 204	208 208	Last thoracic sympathetic ganglia on each side found at autopsy
5	262	229	0	+	204 228	234 252	Left superior cervical ganglion removed. At autopsy splanchnic traced to left adrenal
6	143	61	Not done Adrenals tied		180 173	195 180	Celiac ganglia removed
7	262	229	0 (?)	+	192 203	210 205	Celiac ganglia removed
			Adrenals tied				

* Left side was always sympathectomized first.

Shielded silver-wire electrodes, connected to a Harvard inductorium (3 volts in the primary, 12 to 6 cm. secondary coil distance), provided tetanic induction shocks. A carotid cannula leading to a Hürthle manometer allowed the arterial pressure and heart rate to be recorded optically on rapidly moving film or on kymograph paper.

If the adrenal glands were to be excluded, each one was lifted and tied off in mass.

Anatomical dissections were carried out at the conclusion of each experiment.

Results. Data were secured on 7 cats. Sympathetic regeneration was considered to have taken place when 1, stimulation of the cervical trunk led to retraction of the nictitating membrane and dilatation of the pupil; 2, afferent stimulation elicited an acceleration of the heart.

Stimulation of the cervical chain in the 6 animals studied elicited prompt sympathetic reactions from the nictitating membrane and pupil on one or both sides. Except in cat 7 (table 1) the pupil on the left dilated to a greater extent than the right (the first stage of sympathectomy was always performed on the left side).

The heart rate was counted before afferent stimulation. The highest rate obtained during or after excitation was recorded. In 4 cats no cardiac acceleration followed faradization of the central stump of a somatic afferent nerve. In the remaining 3 animals (nos. 5, 6 and 7) the heart speeded by 15 to 30 beats per minute. Autopsy of cat 5 (table 1) revealed a definitely regenerated splanchnic nerve which was traced to the adrenal gland on the left side. In cat 7, the acceleration of 18 beats was abolished by ligation of the adrenal glands. The same procedure reduced the speeding from 15 to 7 beats per minute in cat 6.

The anatomical investigations, except in a few cases, yielded little of value. The presence of much scar tissue renders even a careful dissection of doubtful worth unless a painstaking histological study is performed. We had the impression that the cervical sympathetic trunk seemed smaller than normal. Occasionally an isolated sympathetic ganglion was found at autopsy. It is of some interest to note that in the one instance where the splanchnic was anatomically visible (cat 5) only a relatively small segment of the nerve was removed at operation, while the lower portion was left.

Discussion. It is known from the work of Langley (1897), Maes and Simeone (1938), and Hinsey, Phillips and Hare (1939) that, under optimal conditions, destroyed preganglionic nerves regenerate and eventually make functional connection with the structures they normally innervate. This is not, however, exactly the same problem as the one studied in the present experiments, for in our animals conditions were far from favorable for regrowth, since, in order to prevent regrowth, large sections of the sympathetic chain and the ganglia were removed.

The fact that even under adverse conditions sympathetic fibers may eventually grow into connections which permit their usual effects emphasizes the necessity of controlling for this condition (as Hinsey, Phillips and Hare have remarked) in long-term experiments on sympathectomized animals. Also from our and previous investigators' experience, the suggestion may be urged that, in order to keep regeneration at a minimum, the greatest possible preganglionic destruction should be practiced.

The most logical explanation of the cardio-acceleration induced by afferent nerve stimulation is the reflex release of adrenaline; for the speeding is completely or partially abolished by ligation of the adrenal glands. The additional contingency exists that preganglionic nerves may have reached the inferior cervical ganglion and there made synapse with some postganglionic cardiac accelerator fibers. Also, it is not impossible that the small amount of sympathin capable of being liberated from the head region could have exerted an accelerant action.

SUMMARY

1. Seven cats, sympathectomized from the stellate to the pelvic ganglia, were studied in acute experiments at varying times after the final surgical intervention.

2. Stimulation of the cervical sympathetic trunk in every case (only 6 cats tested) caused dilatation of the pupil and retraction of the nictitating membrane on one or both sides (table 1).

3. After double vagotomy, afferent nerve stimulation caused no cardiac acceleration in 4 animals. Increase of the heart rate occurred in the remaining 3 cats (table 1). Possible explanations of the acceleration are given (p. 466).

4. The experiments emphasize the necessity of controlling for sympathetic regrowth in long-term experiments, and of practicing as radical sympathetic excision as possible.

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REFLEX CARDIAC ACCELERATION AND LIBERATION OF SYMPATHOMIMETIC SUBSTANCES IN UNANESTHETIZED DOGS DURING ACETYLCHOLINE HYPOTENSION[1]

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Intravenous injection of a selected dose of acetylcholine has been chosen as a controllable method of activating in normal dogs the various mechanisms which compensate for lowered blood pressure. As a result of the rapid destruction of the acetylcholine there is minimal interference with the compensatory reflexes initiated by the fall in blood pressure or with the auto-assay of the neurohormones produced.

The phases of the compensation for acetylcholine hypotension in the unanesthetized dog which have been studied are 1, the reflex cardiac acceleration; 2, reflex liberation of adrenine, and 3, liberation of an intestine-inhibiting substance elsewhere than from the adrenal medulla. The evidence for adrenine in circulation after administration of acetylcholine has been reviewed by Herman et al. (1), who have presented evidence that direct action of acetylcholine on the adrenal glands can be one of the mechanisms involved. Assay of blood from the adrenal veins of normal dogs following acetylcholine administration has indicated a marked increase in the adrenine content (2). In a review of the literature concerning effects of acetylcholine on intestinal motility, Zimmerman et al. (3) have indicated the contradictory results obtained. Dale (4) was aware of the relative impotency of intravenous acetylcholine in producing hypermotility of the intestine in situ as compared with its potency for the isolated intestine. Moreover, he found that acetylcholine was more potent as a cardio-inhibitor than certain choline derivatives which were more effective than acetylcholine in producing increased intestinal motility.

METHODS. Dogs were prepared so that effects of acetylcholine could be determined on blood pressure, rate of the innervated heart, rate of the denervated heart, and motility of the innervated and of the denervated intestine. The animals were under near-basal conditions and received no drugs during the experiment except the acetylcholine.

To determine the effect of acetylcholine on the blood pressure of un-

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anesthetized animals with cardiac innervation intact the spinal cord was transected at the upper lumbar or lower thoracic level by an aseptic operation. Four to eight days after the operation blood pressure was recorded from the femoral artery cannulated at a level just above the knee.

The heart rate was determined by electrocardiographic records from normal dogs and from dogs having had operations designed to denervate the heart. Denervated hearts were prepared by a series of three operations described by McIntyre (5), which result in removal of the sympathetic trunk on each side from the stellate to the fifth thoracic ganglion inclusive, section of the right vagus just below the recurrent laryngeal nerve, and section of the left vagus nerve in the neck.

Effects on the motility of the innervated and denervated intestine were determined by the balloon-mercury-manometer method from Thiry or Thiry-Vella fistulae. Fistulae were prepared and denervated as previously described (6). Four of the animals with denervated fistulae also had their adrenal medullary tissue removed by cautery. One dog with a Thiry fistula also had its coeliac ganglia removed.

Solutions containing 1 or 2 mgm. of acetylcholine hydrochloride per cubic centimeter were prepared by diluting acecoline (Anglo-French Drug Co.) with physiological saline. Precautions were taken to prevent any hydrolysis of the acetylcholine prior to its introduction into the vein.

Results. I. *Effects of acetylcholine on the blood pressure and heart rate of unanesthetized animals.* Six experiments were done with two chord-transected dogs for the purpose of determining the exact time of onset, the rate, and the degree of the fall in blood pressure resulting from an injection of acetylcholine into the radial vein. Doses of 0.03 to 0.06 mgm. per kilo injected into dogs weighing 8 and 9 kilo produced a fall in blood pressure which began within 6 to $7\frac{1}{2}$ seconds after the beginning of the injection. The maximal depression of blood pressure occurred within 2 to 6 seconds after the beginning of the fall. The decrease in blood pressure varied from 38 to 68 mm. of Hg, and the average rate of decrease was 8 to 18 mm. of Hg per second. Blood pressure returned to normal within $\frac{1}{2}$ to 3 minutes after the beginning of the fall.

Effects on the rate of the innervated heart following rapid injection of 0.03 to 0.30 mgm. of acetylcholine per kilo were determined on nine normal dogs by means of electrocardiographic records. Analysis of three typical records and averages of 23 records from these dogs are given in section one of table 1. The heart beats were counted in successive five second intervals following the beginning of the acetylcholine injection and are expressed in the table as rate per minute calculated from the five second intervals.

Of the 23 records there were 11 in which onset of cardiac slowing could be sharply defined. In 7 of these cases the onset of slowing was 6 to 7

seconds after the beginning of the injection. The onset of cardiac acceleration could be determined in every case. Typically, cardiac acceleration began within 7 to 8½ seconds after the beginning of the acetylcholine injection. In all but one experiment acceleration began within 13 seconds, while subsequent experiments indicate that humoral acceleration of the heart is not a factor before at least 13 seconds. It is evident from these time values that cardiac acceleration commonly began within one to two seconds after the beginning of the fall in blood pressure. The maximal acceleration of the heart rate occurred within the five to ten second period after the beginning of the acceleration, and a maximal heart rate 71 to 292 per cent above the resting rate was attained within the 15 to 25 second period. The rate dropped to normal in 50 to 60 seconds and then frequently showed a mild secondary rise.

Hall (7) has described the effects on the electrocardiogram and reported tachycardia in experimental animals following subcutaneous or intravenous injections of acetylcholine. In our experiments the smallest doses gave a record of pure acceleration. Since fall in blood pressure results from this dosage before sufficient time has elapsed for peripheral vasodilatation to occur, it is considered that stroke volume must have been reduced. Larger doses produced a brief period of slowing without irregularities, while the largest doses produced ventricular standstill with only slight slowing of the auricular systoles.

II. *Effects of acetylcholine on the rate of the denervated heart.* Denervation of the hearts of four dogs did not prevent them from showing acceleration after intravenous acetylcholine. However, the first evidence of acceleration was 13.2 to 24 seconds after the beginning of the injection; and the increase in rate was only 10 to 55 per cent; the maximal rate was much lower than for the innervated hearts and was attained later. Analysis of some typical individual experiments and averages of 9 experiments are given in section 2 of table 1.

These results indicate that, if denervation of the heart were complete, humoral acceleration of the heart rate must have occurred within as little as 13.2 seconds. Physiological amounts of adrenalin were injected into the venae cavae of nembutalized dogs at the level of the kidney, and it was found that cardiac acceleration could be produced by this method in as little as 7 seconds. Since the fall in blood pressure begins in 6+ seconds, it is possible to explain an acceleration of the denervated heart in 13+ seconds after the acetylcholine injection on the basis of reflex activation of the adrenal medulla during the fall in arterial blood pressure. However, the early humoral acceleration does not allow a sufficient time interval to permit an explanation of the acceleration on the basis of liberation of adrenine by direct action of acetylcholine on the adrenal medulla.

III. *Effects of acetylcholine on the motility of the innervated and dener-*

vated intestine in situ before and after adrenal demedullation. The denervated dog intestine is at least as sensitive to adrenalin as the denervated dog heart (8). Moreover, some difficulties which are encountered in the use of the denervated heart as an indicator for sympathomimetic substances liberated during acetylcholine hypotension are not encountered when the denervated intestine is used as an indicator. Denervation of the intestinal segment is less difficult and less damaging to the health of the animal. The denervation in this case is mainly or solely for the purpose of sensitization of the intestine to adrenalin (8), since there is no indication that the nerves to the smooth muscle of the intestinal wall are acti-

TABLE 1

Change in rate of innervated hearts (section I) and denervated hearts (section II) of unanesthetized dogs after intravenous injections of acetylcholine

EXPERIMENT NUMBER	WEIGHT	MGM. PER KGM.	ONSET OF SLOWING	ONSET OF ACCELERATION	INCREASE IN HEART RATE	"BASAL" HEART RATE	HEART RATE PER MINUTE AFTER THE ACETYLCHOLINE INJECTION CALCULATED FROM SUCCESSIVE 5 SECOND INTERVALS														
							1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th
Section I																					
	kgm.		*sec.*	*sec.*	*per cent*																
III, 1	10	0.10	6½	7½	140	120	132	192	270	288	246	162	120	104	90	99	114	114	126	144	150
IV, 2	11	0.09	6	7½	120	128	150	174	240	272	282	282	234	174	150	138	138	136	144	136	144
IX, 1	14	0.04	6	12	133	108	114	48	144	252	232	216	140	114	102	102	114	102	126	138	144
Average of 23 experiments...					145	105	111	126	195	243	251	229	185	148	125	117	113	111	120	126	125
Section II																					
D.II, 1	9	0.44	7	24	31	115	116	108	96	116	124	126	138	144	150	144	138	134	132	128	120
D.II, 2	9	0.17	6½	20	10	122	120	114	120	128	136	136	132	132	124	122	126	126	126		
D.IV, 1	8	0.13	6½	16½	43	134	134	72	136	150	168	168	180	192	192	192	192	192	188		
Average of 9 experiments ...					32	128	126	101	117	142	152	160	165	168	157	153	149	147	143	135	132

vated during the acetylcholine hypotension. Finally, there is an advantage in auto-assaying neurohormones with two indicators, one of which is stimulated and the other inhibited by adrenalin.

Sixty records of intestinal motility were taken during intravenous injection of acetylcholine into 13 trained dogs. Each dog had either an innervated or a denervated intestinal segment or both. Activity of the innervated and denervated intestine was studied simultaneously in the same animal in some cases; in other animals the activity of the segment was studied before and after the operation for denervation. Responses of the denervated intestine were determined in the same animal before and after removal of the adrenal medullary tissue. The dosage of acetylcholine

was varied between the same limits used in the study of cardiac responses. Twenty-four of the electrocardiographic records were taken simultaneously with the intestinal records.

Both the innervated and the denervated intestine showed a brief period of excitation after the acetylcholine injection. The excitation was followed by a more or less prolonged period of inhibition. The typical effects are illustrated in figure 1. The records are similar to those obtained

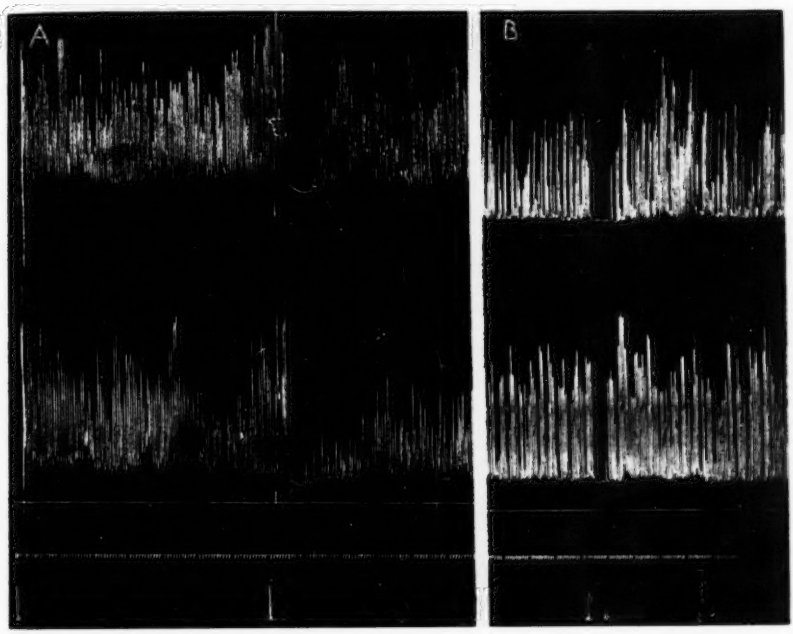

Fig. 1. A. Effect on the motility of denervated (upper record) and innervated (lower record) intestinal segments of 0.1 mgm. per kilo of acetylcholine injected into the radial vein.

B. Effect on motility of the denervated (upper record) and innervated (lower record) intestinal segments of 0.001 mgm. per kilo of adrenalin injected into the radial vein. Time in one minute intervals.

by Harper (9). The onset of the excitation, as manifested by an increase in tonus and/or increased amplitude of rhythmic contractions, typically occurred within the period between 9 and 15 seconds after the beginning of the intravenous injection. The increased motility typically lasted 6 to 9 seconds and was then suddenly replaced by complete inhibition of rhythmic contractions and a decrease in tonus. The onset of both the excitation and the inhibition could be accurately determined in 36 of the

experiments. The inhibitory phase typically began within 6 to 10 seconds after the beginning of the excitation. Definite inhibition lasted from 15 seconds to 3 minutes after its onset and was complete in 37 of the experiments. When records were taken simultaneously from innervated and denervated segments in the same animal, the denervated segment was typically inhibited more than the innervated. This is illustrated in figure 1, which also shows that differential inhibition results from administration of an amount of adrenalin well within the physiological range. All of the responses to acetylcholine injection subsequent to the brief hyper-activity are indistinguishable from those resulting from injection of adrenalin at physiological rates (8). The short duration of the direct effect of the acetylcholine is explicable on the basis of reflex activation of the adrenal medulla at the time when the blood pressure is falling, thus resulting in a discharge of adrenine into the venous system in as little as 6 to 7 seconds after the intravenous injection of acetylcholine. Thus the same minimum time value for the acetylcholine effect to precede the adrenine effect was obtained for both denervated heart and denervated intestine. The cardiac denervation reduced the per cent of increase in the heart rate as well as the maximal rate attained and increased the latent period preceding acceleration after injection of acetylcholine. This is to be contrasted with the unchanged time values and the increased degree of inhibition in the intestine after denervation. These facts indicate that the inhibition of the intestine was purely humoral.

Proof that a part of the intestine-inhibiting substance was liberated from the adrenal medulla during acetylcholine hypotension was completed by repeating the acetylcholine injections in four dogs after bilateral adrenal demedullation. The denervated intestine showed the sequence of excitation and inhibition as before, but the period of excitation was prolonged, and the onset of inhibition was more gradual. The alteration of the response of the denervated intestine to acetylcholine by adrenal demedullation is illustrated in figure 2.

Records from adrenal demedullated animals indicated that the denervated intestine after a latent period was inhibited by a substance originating elsewhere than in the adrenal medulla. Several facts indicate the origin of this substance from adrenergic nerve endings. Widespread activation of adrenergic nerves (vasoconstrictor, cardioaccelerator) occurs during a sharp fall in blood pressure such as was produced by the acetylcholine injections. Adequate time elapsed to allow for the diffusion of the sympathin into circulation and its carriage to the denervated intestinal segment. The action of the substance on the intestine was identical to the action of adrenalin. Finally, the excitatory effect of acetylcholine on the intestine was prolonged and the inhibitory phase not obtained in a dog having its coeliac ganglia removed.

Intravenous injection of acetylcholine seems to afford a controlled method of reflexly activating certain excitatory sympathetic nerves in the intact dog. Failure of acetylcholine hypotension to activate inhibitory adrenergic nerves to the smooth muscle of the intestinal wall indicates that there is not indiscriminate activation of the entire sympathetic nervous system. According to the "two sympathins" theory the sympathin produced at excitatory adrenergic endings differs from adrenalin and lacks inhibitory effects (10). However, "liver sympathin," which was thought to be pure excitatory sympathin, will inhibit the intestine (11). This objection has been answered by Cannon and Rosenblueth (12) with the suggestion that some of the mediator (adrenalin) may enter circulation without combining with the effector substance. Although it appears that

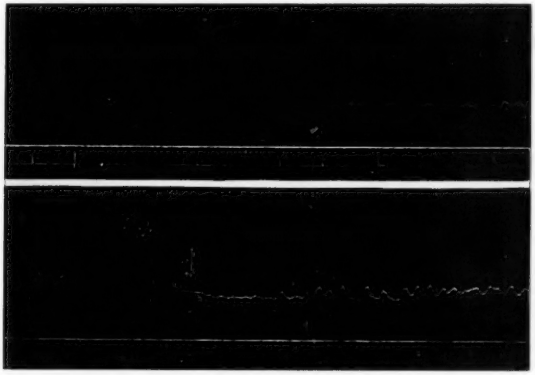

Fig. 2. Effect on the motility of the denervated intestinal segment of 0.10 mgm. per kilo of acetylcholine injected into the radial vein of the same unanesthetized dog before (upper record) and after (lower record) aseptic operations for demedullation of the adrenal glands. Time in 5 second intervals.

the adrenergic nerves activated by a fall in blood pressure are mainly of the excitatory type, the neurohormones produced in the adrenal-demedullated animal are potent inhibitors of the denervated intestine.

Since the well-known mechanisms which compensate for lowered blood pressure are utilized following an intravenous injection of acetylcholine, the responses of a given effector may be predicted on the basis of knowledge concerning these compensatory mechanisms and the direct effects of acetylcholine and adrenalin on the effector.

CONCLUSIONS

Intravenous injection of doses of acetylcholine between 0.03 and 0.30 mgm. per kilo is a method of activating in intact dogs the mechanisms which compensate for lowered arterial blood pressure.

After the injection of acetylcholine the heart beat is weakened or the heart rate is slowed momentarily by direct action of acetylcholine, and a sharp fall in blood pressure results. During the fall in blood pressure the innervated heart is reflexly accelerated and attains, within a few seconds, a rate 70 to 292 per cent higher than basal.

The rate of the denervated heart begins to increase in not less than 13 seconds after the intravenous injection of acetylcholine. This early humoral acceleration is caused by adrenine which is reflexly liberated from the adrenal medullae during the low blood pressure.

Although the above dosage of acetylcholine typically produces a fall in mean arterial blood pressure of more than 50 mm. of mercury, the compensatory mechanisms restore the blood pressure to the normal level in the unanesthetized animal within 30 to 180 seconds.

The first change in the motility of the innervated or denervated intestine after the intravenous injection of acetylcholine is an increased tonus with or without increased amplitude of rhythmic contractions. The excitation, produced by the direct action of the compound on the intestinal smooth muscle, lasts only a few seconds and is replaced by a much longer period of inhibition as a result, in part, of the action of the reflexly produced adrenine. The intestine is not reflexly inhibited during acetylcholine hypotension.

Adrenal demedullation allows a more prolonged direct acetylcholine effect on the denervated intestine, but complete inhibition still occurs. The inhibition in this case is attributable to sympathin liberated during the activity of the adrenergic nerves which are reflexly activated by a fall in arterial blood pressure.

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THE THYMUS-ADRENAL RELATIONSHIP

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Soli, in 1909, was apparently the first to notice that a slight hypertrophy of the adrenals follows thymectomy. Gershon-Cohen et al. (1938) found that the adrenals were consistently enlarged in young male rats whose thymus glands had undergone atrophy after the administration of large doses of x-ray.

Conversely, Rowntree (1935) has found that the administration of thymus extract (Hanson) leads to a hypoplasia of the rat adrenal. On the other hand, Tislowitz and Chodkowska (1936) have reported that the administration of thymus extracts produced somatic growth and adrenal hypertrophy in the rabbit. Autopsy findings, in cases of (human) sudden death, so-called "status lymphaticus," often reveal an enlarged thymus associated with a hypoplastic adrenal (Marine (1928). In fact, one current opinion ascribes the sudden death to adrenal insufficiency rather than to the enlarged thymus. Indeed, the enlarged thymus may well be due to an adrenal insufficiency, for Jaffee (1924), Marine (1924, 1926) and Kitagawa (1927) have reported that bilateral adrenalectomy leads to hyperplasia of the thymus. Pappenheimer, in 1910, pointed out that an enlarged or persistent thymus is frequently associated with the atrophy of the adrenals in Addison's disease. Seitz et al. (1925) found that there was a distinct enlargement of the thymus in young rabbits following adrenalectomy in the parents.

Andersen (1935) confirmed by the more extensive studies of Selye (1937), reported that excessive muscular exercise in the rat was followed by a syndrome whose conspicuous features were enlargement of the adrenals and marked atrophy of the thymus. Selye found that this "alarm-reaction" is non-specific, since it was quickly produced by any noxious stimulus (cold, formalin, morphine, adrenalin, etc.). He further found that adrenalectomy prevented the characteristic thymic changes of this syndrome. It would thus appear that the thymic atrophy is due to an overproduction of the cortical hormone. Indeed, Selye reports that only

[1] This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

cortical hormone and estrone, of the substances he has tried, are capable of producing thymic atrophy in the absence of the adrenals. Further evidence that cortical hormone is probably the active agent in the production of thymus atrophy is given in the recent findings of Evans et al. (1938), that the adrenocorticotropic hormone of the pituitary will produce thymic atrophy in an animal with intact adrenals.

The researches cited above would appear to delineate a picture of mutual antagonism.[2] It would appear that adrenalectomy removes some factor which controls the thymus since the latter enlarges in the absence of the adrenals. On the other hand, if the total cortin in action is increased (by injections or by the more strenuous method of administering some one of the agents which evoke the "alarm-reaction"), the thymus atrophies. The thymus in turn appears to have some effect on adrenal function inasmuch as thymectomy leads to enlargement of the adrenals and the augmentation of thymic products (by injections of thymus extracts) causes a partial adrenal atrophy.

Apart from the sort of antagonism which expresses itself in effects upon the glands themselves, there is some evidence for an antagonistic action upon extrinsic physiological processes. Messini and Coppo (1932) found that thymectomized rabbits exhibited hyperchloremia and decreased cutaneous and connective tissue chlorides; their work being extended by Marconi's (1937) demonstration in the same animal of an increased urinary chloride production after thymectomy, and of hypochloremia following the administration of thymus (or lymph gland) extracts.[3] Parhon and Werner (1936) showed that thymus extract (Hanson) decreased the serum potassium. Since, as is well known, adrenalectomy leads to hypochloremia and an increase in serum potassium, and administration of cortin to normal animals increases serum chloride and decreases serum potassium, the results of more thymus effect and less adrenal, or more adrenal effect and less thymus, would appear to be similar. However, there is something anomalous about the fact that both thymectomy and adrenalectomy produce an increase in urinary chlorides.

EXPERIMENTAL. The experiments herein reported were undertaken to test the thymus-adrenal relationship, under the hypothesis that if the thymus is removed, the course of adrenal insufficiency following adrenal-

[2] The work of La Grutta and Avellone (1933) seems, on the other hand, to show that a "mutual aid" relationship holds to some extent, for their adrenalectomized cats lived longer when given thymus extracts. However, these extracts were prepared by Hartman's method for the preparation of cortin and conceivably owed their efficacy to a content of cortin-like compounds. This possibility is strengthened by the fact that cortin injections increased, even further, the survival of the adrenalectomized animals.

[3] It is interesting to note that only the thymus extracts were effective upon normal human subjects.

ectomy might be expected to be altered. The cortin used was kindly supplied by Parke, Davis and Company through the courtesy of Drs. Oliver Kamm and D. A. McGinty.

Two series of animals were used. Those of the first came from the strain of hooded rats maintained in this laboratory, those of the second being male albino rats obtained from Mr. Frazier[4] of Detroit. They were all fed our normal colony diet of Ti-O-Ga dog chow with a bi-weekly supplement of lettuce and tap water ad libitum. The cortin was injected twice daily.

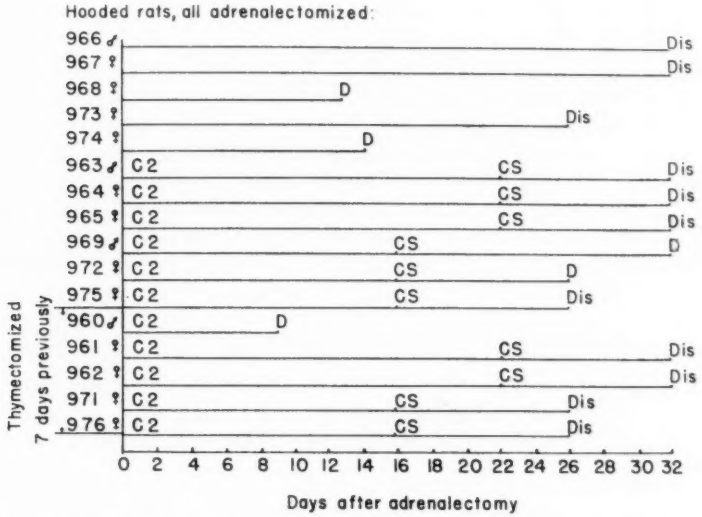

Fig. 1. Survivals of adrenalectomized rats. *D*—Died. *Dis*—Discarded. *C2*—Two rat units cortin daily (in two doses) until further notation. *CS*—Cortin injections stopped.

The results with the hooded rats are shown in figure 1. It will be noticed that only two of the five untreated adrenalectomized animals died during the experimental period. Likewise, only two of the six adrenalectomized animals given two rat units of cortin daily died after the cessation of the injections. Further, only one of the five thymoadrenalectomized animals died during the course of the experiment. It is of interest to note that one of the surviving females had sufficient accessory adrenal tissue to permit a successful pregnancy and lactation after she had been discarded from the experiment.

Figure 2 shows the survival periods and other pertinent data for the Frazier animals, while figure 3 shows their growth curves. It should be

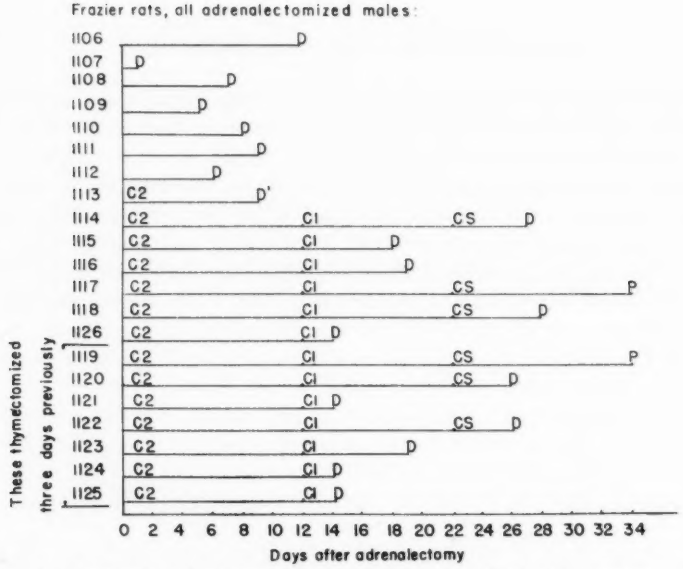

Fig. 2. Survivals of adrenalectomized rats. *D*—Died. *P*—Autopsy. *D'*—Died of respiratory infection. *C1*—One rat unit cortin daily (in two doses) until further notation. *C2*—Two rat units of cortin daily (in two doses) until further notation. *C.S.*—Cortin injections stopped.

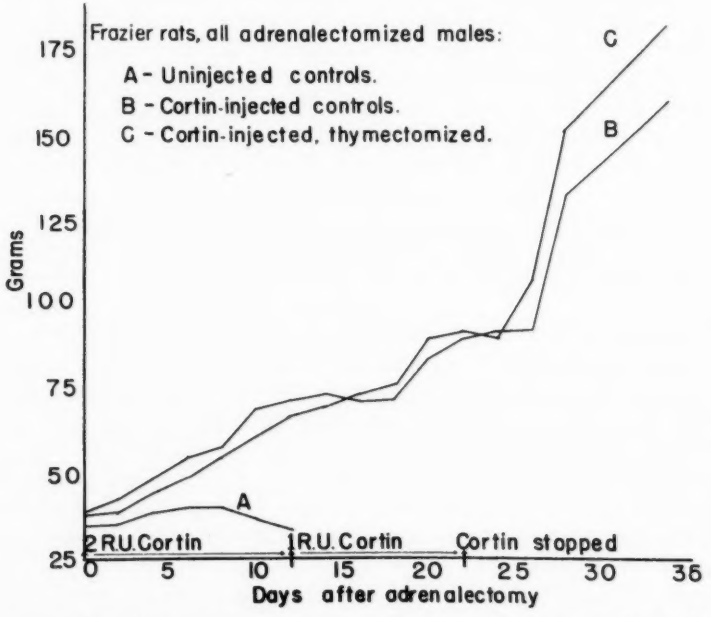

Fig. 3. Growth curves for Frazier rats, all adrenalectomized males: with and without maintenance doses of cortin.

noted that all of the adrenalectomized controls were dead by the twelfth day following adrenalectomy. Animal 1113 died of an acute respiratory infection on the seventh day following adrenalectomy despite two rat units of cortin daily. As can be seen from figure 3, the two rat units of cortin, used initially, adequately maintained both the adrenalectomized and the thymo-adrenalectomized animals. However, when the change was made to one rat unit of cortin daily, only half of each group survived. One animal in the thymo-adrenalectomized group and one in the adrenalectomized group survived the cessation of cortin treatment and were found to have macroscopic accessory tissue when autopsied 34 days after adrenalectomy.

SUMMARY AND CONCLUSIONS

Evidence has been presented from the literature which shows that some sort of reciprocal relationship exists between the thymus and the adrenal glands. In view of this evidence experiments were undertaken to ascertain whether thymectomy would alter the course of adrenal insufficiency in the bilaterally adrenalectomized rat.

Thymectomy failed completely to produce any such effect, as is shown by both the growth curves and the survival periods following bilateral adrenalectomy. It thus appears that despite any effect the thymus may have upon the function of the adrenals, or upon adrenal-controlled physiological processes, it is quite inadequate to cope with the acute situation in the adrenalectomized animal.

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[4]This strain is used by Parke, Davis and Company for purposes requiring adrenalectomy, since they are of known and constant susceptibility to this operation—apparently lacking in accessory adrenal tissue. The difference in survival rates after adrenalectomy in animals from our own colony and from the Frazier stock confirm the extended study of Gaunt, Gaunt and Tobin (1935) on various strains of rats.

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THE RELATION OF RENIN TO THE ADRENAL GLAND[1]

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In 1898 Tigerstedt and Bergman (1) described in the normal kidney a pressor material which they named renin. It is a thermolabile protein-like substance, limited in occurrence to the renal cortex and has a generalized vasoconstrictor action which appears to be independent of the central nervous system. Under certain conditions it may be found in increased concentrations in the injured kidneys of animals with experimental renal hypertension (2, 3, 4). Since this type of hypertension is either prevented or poorly maintained in the absence of adrenal glands (5–8), it seemed of importance to study the relation of the substance renin to the adrenal glands.

Two phases of the problem were considered: I. Does removal of the adrenals affect the concentration of renin in the kidney? II. Does adrenalectomy alter the response of the animal to renin?

I. Effect of adrenalectomy on the concentration of renin in the kidney. Cats were used as experimental animals and all operations were carried out under nembutal anesthesia. Both adrenals were removed at a single operation. Post-operatively the animals were given daily doses of sodium chloride parenterally, but no other medication. They either died or were sacrificed at varying intervals (2 to 10 days) after operation and the completeness of adrenalectomy was checked at autopsy. The kidneys were removed, weighed, and extracts made from alcohol-insoluble fractions (9). The effect of these extracts on the blood pressure was tested on trained unanesthetized dogs with carotid artery loops (10). Four test dogs of approximately the same size were used throughout these experiments. The solution to be tested (equivalent to 0.5 gram of dry powder) was injected intravenously after the level of the resting systolic blood pressure had been determined. The peak of the rise in blood pressure usually occurred in 1½ to 5 minutes and was maintained for several minutes. The average of all of the readings for a period of 1 minute at the peak of the curve was determined, the difference between this figure and the initial pressure level being considered as the *maximum pressor effect*. Control observations were made on extracts of kidneys removed from normal unoperated cats kept under similar conditions. Palpation of the exteriorized carotid artery often yields false high

[1] A preliminary report of this study was read at the Meeting of the American Society for Clinical Investigation, May 1, 1939.

[2] This investigation was aided by the Richard and Ella Hunt Sutro Fund.

readings so that the absolute figures for blood pressure are unreliable, particularly in the higher ranges. The method however is adequate for determining qualitative changes.

The maximum pressor effect of extracts prepared from 34 normal kidneys ranged between 3 and 82 mm. of mercury, the average being 36 mm. of mercury. The maximum pressor effect of extracts of kidneys removed from 20 adrenalectomized animals varied between 15 and 117, the average being 58 mm. of mercury.

Although there is a wide range of variation, it may be seen that the amount of pressor substance is certainly not reduced in kidneys of adrenalectomized animals. In fact it seems to be present in somewhat higher concentrations than in kidneys removed from normal animals.

Because of this marked variation in concentration of pressor material in different cats it seemed advisable to test the changes in the same animal before and after adrenalectomy.

In 12 cats, accordingly, one kidney was removed and used as a control, and at the same time the adrenal on that side was excised. After varying intervals the second adrenal was removed. Within a few days the animal either died or was sacrificed, and the second kidney was removed. The two kidneys from each cat were extracted in the manner previously described and comparable doses of the extracts were tested on the same test dog. The removal of one kidney together with the adrenal appears to shorten the survival period. None of the 12 cats in this series lived longer than 4 days, the average being $2\frac{1}{2}$ days after removal of the second adrenal gland. The maximum pressor effects of extracts prepared from the remaining kidney as compared with those of the control kidney were greater in 9, unchanged in 2, and slightly diminished in only 1 animal.

Control observations were made in 16 cats on the effect of unilateral nephrectomy alone on the concentration of renin in the remaining kidney, the adrenals being left intact. The maximum pressor effect of the extracts prepared from the remaining kidney as compared with that of the control kidney of the same animal was greater in 5, unchanged in 5, and diminished in 6 instances.

Neither in this nor in the previous series is there any evidence of reduction in the amount of renin in the kidneys as a result of adrenalectomy. Whether or not the observed increase in concentration is significant cannot be stated on the basis of this small series. Changes in concentration due to dehydration which often accompanies adrenal insufficiency may conceivably play a rôle but have been minimized in these experiments since the assays were based on the dried powder.

II. The response to renin following adrenalectomy. The response to standard doses of renin and other pressor substances was recorded in a group of dogs prior to and at varying intervals following removal of one or both adrenal glands.

Bilateral adrenalectomy was performed in two stages by a lumbar extraperitoneal approach, using nembutal anesthesia. Sodium chloride and citrate by stomach tube were given daily except when withheld for purposes of the experiment. Adrenal cortical hormone[3] was administered usually on the day of the removal of the second adrenal, and subsequently when signs of adrenal insufficiency developed. For control studies tyramine hydrochloride was chosen as the pressor substance because it has relatively few undesirable side actions and resembles renin in its effect on the blood pressure. In some dogs additional observations were made, using amphetamine and epinephrine. The material to be tested was injected intravenously into the unanesthetized dog, the blood pressure being recorded in the forelimb by a modification of the auscultatory method described by Allen (11) and applied with some modifications by Ferris and Hynes (12) and by Collins (13). All of these investigators used the hind-limb.

The forelimb is superior to the hind-limb in that the more cylindrical shape permits the cuff to remain in place without the addition of complicated supporting straps. It is advisable to select dogs with readily palpable arterial pulsations. A thin rubber sleeve 20 cm. in length (the upper part of a surgical glove is quite satisfactory) is placed loosely around the forelimb above the elbow joint and a small blood pressure cuff, measuring 7 x 15 cm., is applied snugly over it. The protruding lower half of the rubber sleeve is doubled back over the cuff and is held in place by a circular band of adhesive tape. A small diaphragm-type stethoscope is applied and secured in place by adhesive tape directly over the artery below the cuff. We have found this method to give reliable readings for systolic pressure as evidenced by consistent readings in individual animals from day to day,—checks against simultaneous observations by direct arterial cannulation and by the finding of characteristic curves to known vasoactive substances such as epinephrine, renin, and nitrites. Diastolic pressure readings are not as reliable in many animals because of the indistinct end-points. Only the systolic readings were used to determine the pressor effect.

RESULTS. The effects of renin and of tyramine were compared before and after removal of the adrenal glands in 10 dogs. Removal of one adrenal did not alter the response to renin or tyramine. Bilateral adrenalectomy was followed in 9 animals by a marked decline in response to renin and by slight diminution or no change in response to tyramine. In one dog the falling off in the actions of renin and tyramine were about the same in magnitude. In two dogs amphetamine (benzedrine) and epinephrine were used in addition to tyramine as test substances, inducing in each instance good pressor effects at a time when little or no response was elicited by renin. The marked decrease in sensitivity to renin did not occur immediately, but after an interval of 3 or 4 days, and in several instances as long as 14 days after complete adrenalectomy, and could be precipitated by withdrawal of supportive saline therapy.

Administration of adrenal cortex extract was followed in every instance by restoration toward the normal response to renin. Withdrawal of the extract resulted in a gradual and progressive decline in the effect of renin. These changes in sensitivity to renin effected by giving or withholding

[3] We are indebted to Wilson and Co. for a generous supply of adrenal cortex hormone.

adrenal cortex hormone were induced in repeated cycles in the same animal. No consistent fluctuation in the actions of tyramine, benzedrine, or epinephrine were observed during these cycles. The day to day record in a representative animal is shown graphically in figure 1.

The distribution of the maximum pressor effects for renin and tyramine is shown in table 1, which includes *a*, all the readings prior to operation; *b*, those after removal of one adrenal, and *c*, after complete adrenalectomy.

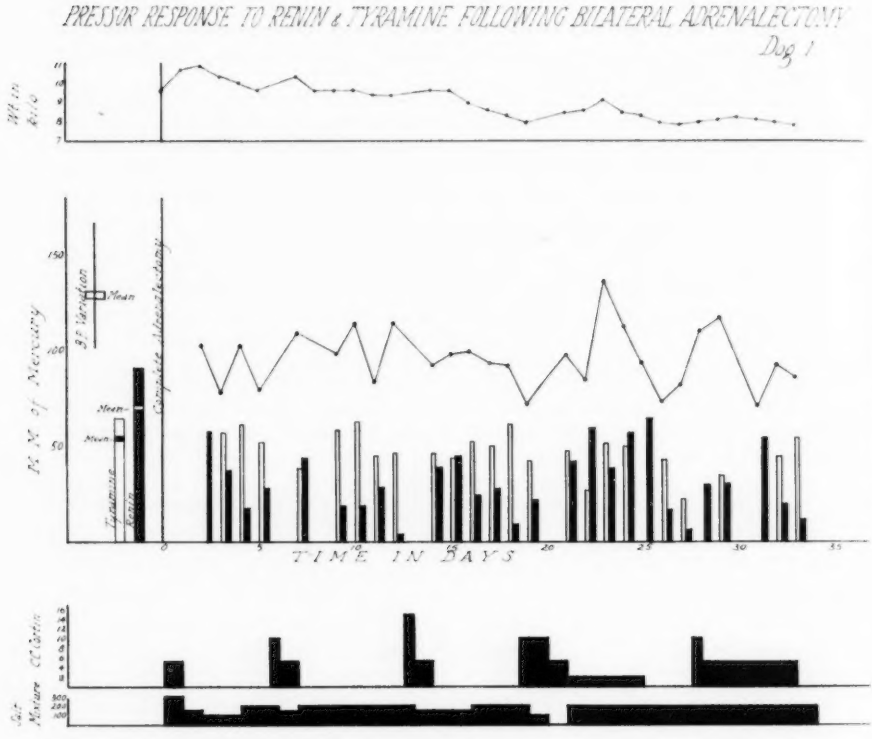

Fig. 1

The last group was subdivided into two categories based on the use of substitution therapy: 1, all readings obtained on days on which the dog received adrenal cortical hormone; 2, all readings after a period of 3 or more days without cortical extract. A statistical analysis indicates that there was a significant decline in the response to renin following adrenalectomy which was reversed in part by substitution of adrenal cortical extract. The effect of tyramine under the same conditions was either unchanged or

only slightly reduced. Comparison of the responses to renin prior to operation with those following total adrenalectomy without administration of adrenal cortical hormone yielded a probability ratio (P.R.) of 6.0, while the P.R. for tyramine in the same groups was 1.0.[4]

Discussion. Our observations in dogs are in agreement with those recently published by Williams, Diaz, Burch and Harrison (14), using the rat as a test animal. They found, as we did, that adrenalectomy lowered the sensitivity of the animals to renin but did not reduce the concentration of renin within the kidneys.

TABLE 1

Distribution of maximum pressor effects of renin and tyramine

	RENIN					TYRAMINE				
	Number of tests	Smallest effect	Largest effect	Mean	Standard deviation from mean	Number of tests	Smallest effect	Largest effect	Mean	Standard deviation from mean
		mm. Hg	*mm. Hg*	*mm. Hg*			*mm. Hg*	*mm. Hg*	*mm. Hg*	
Normal dogs before operation	44	41	101	61.4	3.1	33	50	110	71.3	1.9
After unilateral adrenalectomy	55	36	112	63.5	2.5	58	21	106	67.1	0.9
After bilateral adrenalectomy—No adrenal cortex extract	51	0	85	35.7	3.2	47	22	114	61.7	2.8
After bilateral adrenalectomy—Adrenal cortex extract given on day of test	28	11	97	45.9	4.6	22	26	124	55.6	4.6

The loss of response to renin usually coincided with the appearance of evidence of adrenal insufficiency but not necessarily with a decline in the dog's blood pressure. On the other hand, normal responses were obtained in some dogs whose systolic blood pressures had fallen to shock levels. No correlation was observed between the response to renin and the level of the animal's blood pressure, suggesting that renin is not an important factor in the maintenance of normal blood pressure. Since the pressor effect of renin does not diminish immediately after removal of the adrenal glands, but only after a prolonged time interval, one may assume that it

[4] We wish to thank Dr. F. Hollander for his aid in the statistical analysis.

is not dependent on some intermediate metabolic change which renin must undergo within the adrenal gland. It suggests rather that a substance formed in the adrenal glands sensitizes the vessels to the action of renin. The loss of sensitivity after adrenalectomy appears to be specific for renin since ability to respond to other pressor substances is well maintained. This is true both for amphetamine, whose action is predominantly on the peripheral resistance (15), as well as for tyramine whose pressor effect is due in part at least to an increase in cardiac output (16, 17).

Renin has been suggested as the hypothetical stimulating substance, the increased production (or diminished destruction) of which may be responsible for the elevation of blood pressure in experimental renal ischemia. There is general agreement that this type of hypertension, if it develops at all, is poorly maintained in the absence of the adrenal cortex or adequate substitution therapy (5–8). A plausible explanation for the failure to maintain an elevated level of blood pressure in adrenalectomized animals with renal ischemia is suggested by the demonstration of the loss of sensitivity to renin following removal of the adrenal gland. This relationship affords additional evidence in favor of the hypothesis that renin is intimately concerned in the mechanism of the hypertension due to renal injuries.

CONCLUSIONS

1. The concentration of renin in the kidneys of cats was not reduced by adrenalectomy.

2. Following removal of the adrenal glands in dogs there was a gradual diminution in response to renin. This was restored in part by substitution of adrenal cortical hormone.

3. The results suggest an explanation for the effects observed after ablation of the adrenal glands in animals with experimental renal hypertension, and lend support to the hypothesis that renin is intimately concerned in the mechanism of this type of hypertension.

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A METHOD FOR THE SIMULTANEOUS RECORDING OF FOCAL CEREBRAL BLOOD FLOW, pH, ELECTRICAL ACTIVITY AND BLOOD PRESSURE

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The electrical activity, pH and blood flow of focal areas of the cerebral cortex have been shown to be important indices of certain aspects of cerebral function. With the use of small glass electrodes developed by Nims (1), Dusser de Barenne, McCulloch and Nims (2) have shown important relations to exist between the functional activity and the pH of a given cortical area. Penfield, von Santha and Cipriani (3) have shown that there is a focal increase in blood flow to a given cortical area associated with an increase in its activity. Numerous authors (4) have shown the electroencephalogram to be an important sign of cortical function. Little is known, however, of the exact interrelationship between these three variables. A method of recording them simultaneously and conveniently with a minimum of time lag is presented here.

The recording of pH, blood flow and blood pressure requires the use of some direct current measuring device of fairly high sensitivity (especially for blood flow). By this method the records were taken with an ordinary condenser coupled amplifier and ink writer as used for the recording of electroencephalograms. The inputs to the amplifiers were shorted about 16 times per second, so that the record was in the form of repeated deflections, the heights of which were proportional to the D.C. input voltage. This principle was described by Wohlisch (5) for the thermoelectric measurement of muscle heat. More recently Loomis (6) has employed it for the measurement of D.C. potential changes in the brain.

A photograph of the interrupter is shown in figure 1. The unit is built up from a variable speed motor *M* and an automobile distributor *D*. The essential part is a four sided cam *C* so cut that it produces a longer make than break, this gives a direction to the deflections as suggested by Loomis, so that reversals in their direction can be clearly interpreted. Bearing on this cam are three breaker arms, *1*, *2*, *3*, carrying silver contacts. These make and break with three stationary silver contacts so that all rubbing is eliminated. Each pair of contacts is shunted by 10,000 ohms and the whole device is carefully shielded.

In recording pH the voltage changes at the glass electrode are fed to a microvolter as described by Nims and then to the interrupted amplifier input. The use of the microvolter was necessitated by the low resistance

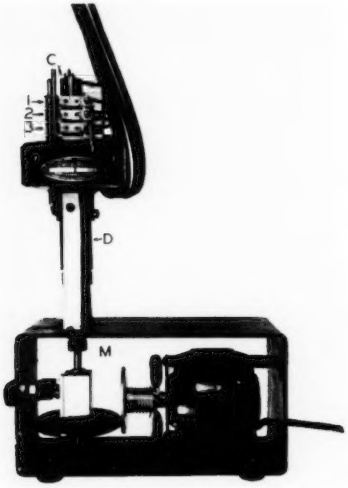

Fig. 1

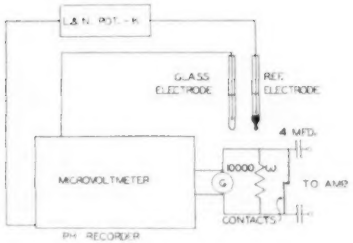

Fig. 2

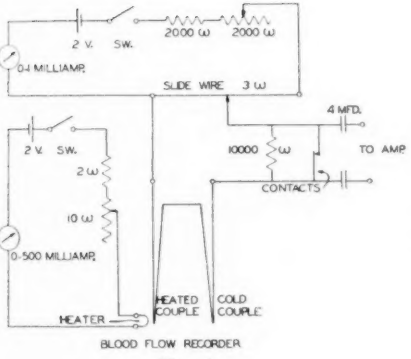

Fig. 3

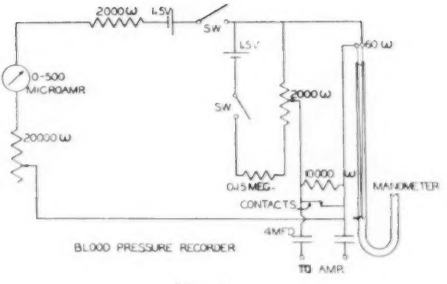

Fig. 4

shunt across the contacts which was essential for their quiet operation. The circuit is shown in figure 2.

The blood flow recorder, shown in figure 3, makes use of a heated thermocouple similar to that described by Gibbs (7). Blood pressure was converted into voltage changes by sealing a loop of constantin wire into a

mercury manometer so that variations in the level of mercury would vary the resistance of the loop and consequently the voltage across the amplifier inputs, as shown in the circuit diagram (fig. 4).

In all recordings potentiometers were used to balance out initial d.c. components, so that only changes from the steady state were recorded.

The electrical activity of the cortex was recorded from a fine wire in-

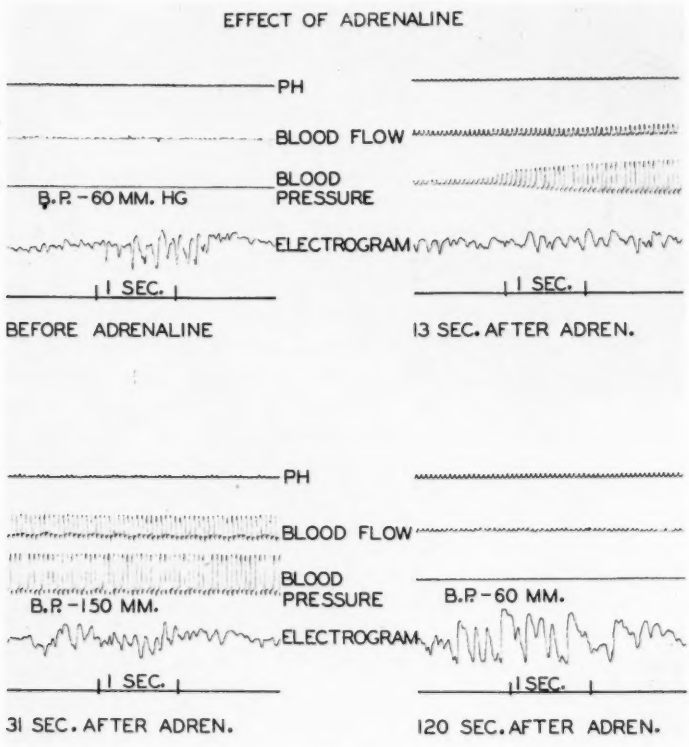

Fig. 5

corporated in the thermocouple and from the reference pH electrode. The voltage in this case was not interrupted.

The value of the method is illustrated by two experiments, conducted on the exposed cortex of a cat under dial anesthesia. The records in figure 5 show the effect of injection of 0.5 cc. of 1/1000 adrenaline intravenously. Here there is increased blood pressure, increased blood flow and some increase in electrical activity with little change in pH. In figure 6 the respiration was stopped for three minutes and then hyperventilation immediately

started. First there was a fall in pH accompanying the stoppage of respiration. With hyperventilation the pH returned to the normal level and subsequently increased. In the first phase there was a slight increase in blood flow and slowing of the brain waves. With hyperventilation there was no appreciable change in either blood flow, blood pressure or cortical activity.

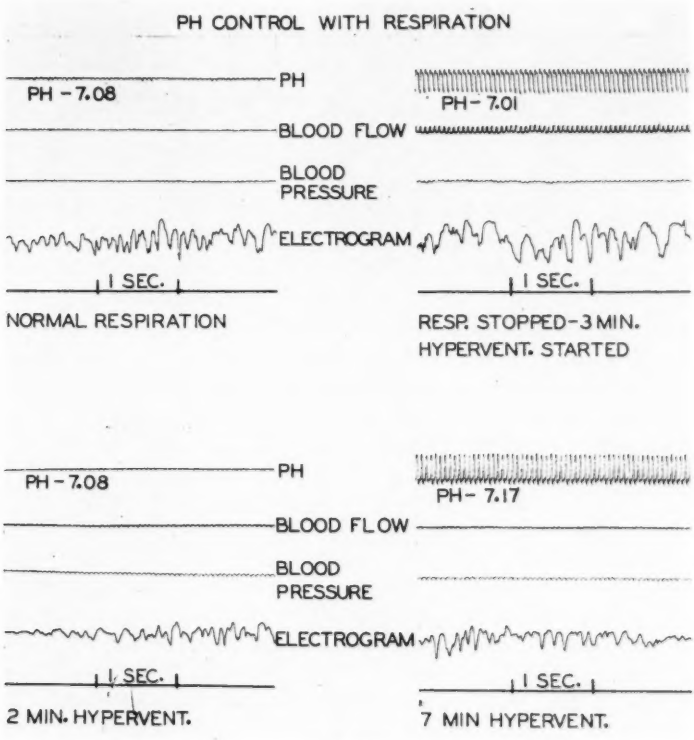

Fig. 6

Further studies with this technique will be reported at a later date. It is presented at this time to show a simple method of adapting standard condenser-coupled amplifiers and ink-writing recorders to the simultaneous measurement of a variety of physiological variables. Direct current voltage changes of the order of 5 microvolts are clearly indicated from a quiet base line. There is no drift in the recording system and the type of record obtained is particularly suited to physiological experiments where measurements are to be made above and below an adjustable zero level.

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THE RELATION OF DIET TO THE RESTITUTION OF THE GONADOTROPIC HORMONE CONTENT OF THE DISCHARGED RABBIT PITUITARY

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In recent years gonadotropic extracts have been prepared from several foodstuffs (1, 2, 3) but there has been no evidence presented to show that these gonadotropic materials are of physiological significance. Although we do not know what raw materials are required for the elaboration of the pituitary hormones, the chemical nature of the active fractions suggests that they might be derived, ultimately, from dietary protein. In the rat restriction of dietary protein, quantitatively or qualitatively, leads to an abrupt cessation of the vaginal estrous cycle and of ovulation (4, 5, 6, 7). There is one report that pituitary implants are capable of stimulating the ovaries of rats suffering from the anestrus of protein restriction (5), but the evidence is not convincing that the condition is actually due to pituitary hypofunction.

If it were possible to produce a true pituitary hypofunction by dietary deficiency, one might then be able to determine the physiological and nutritional significance of the foodstuffs which yield gonadotropic extracts. The success of such experimentation would depend upon our ability to measure the rate of formation of the gonadotropic hormone, and there is at hand no adequate method for such measurement. The very rapid changes in the rabbit pituitary, however, offer a means of approximation. It will be recalled that the gonadotropic hormone of rabbit pituitary is almost completely discharged by the act of coitus (8, 9) and that for the first 4 to 6 days of pseudopregnancy the hormone content of the gland does not increase significantly; i.e., the amount of hormone formed is not much in excess of that released. Between the 6th and 10th days of pseudopregnancy, the hormone content rises sharply toward the initial estrous level, and this increase in hormone content must represent the excess of the amount formed over the amount released from the gland. If we may assume that the rate of release of gonadotropic hormone into the blood stream is modified chiefly by the ovaries, then we might also assume that the rate of gonadotropic hormone secretion (into the blood stream) would be the same in groups of animals with ovaries in precisely the same

physiological condition. Consequently, any difference in the rate of formation of the hormone should be reflected by a change in the rate at which the excess is built up between the 6th and 10th days of pseudopregnancy.

On the basis of such considerations the present experiments were designed to determine the influence of protein restriction on the rate of restitution of gonadotropic hormone in rabbit pituitaries which had been discharged by coitus.

METHODS. The experimental animals were either post-partum, or post-pseudopregnant rabbits in good health and consuming normal quantities of the stock ration (diet 16) daily. Groups of such animals were transferred to the experimental rations for varying periods of protein deprivation. Eight days before the termination of each dietary experiment all animals of the group were mated with a vasectomized male. This plan was followed because the 8th day of pseudopregnancy is a time when the pituitary gonadotropic content is rising most sharply (autumn) or has just about recovered the estrous level (spring). At autopsy, any animal which had failed to ovulate (there were a few) or which showed any genital infection or other abnormality was discarded. Likewise, during the course of these experiments we discarded any animal which developed respiratory infection or diarrhea.

The pooled glands of each experimental group (varying from 3 to 9 animals each, but usually five or six) were placed in acetone immediately, and dried and assayed a few days later by a method recently described (10). The values obtained were compared with the assays of pooled glands of animals on the stock ration, killed on the same day of pseudopregnancy and at the same time of the year. This precaution of parallel controls was found necessary because of the seasonal variation in the rabbit pituitary (9).

The diets employed are shown in table 1. They were fed in the form of a thick paste, the ration for each animal being weighed out daily. The food consumption was determined by weighing the contents of the feed bowl on the following morning, no allowance being made for evaporation since this had been shown to be negligible. Wastage also was negligible except in the case of a few animals and this could easily be detected in the clean shavings beneath the mesh flooring of the cage.

As a guide to the course of protein starvation repeated determinations were made of urinary nitrogen excretion in some of the animals. Just preceding each 24 hour collection the residual bladder urine was expressed by pressure on the lower abdomen. At the end of the 24 hour period the bladder was again emptied by pressure and the resulting urine added to that collected in the metabolism cage, the total volume so obtained varying from 150 to 350 cc. The urine, plus the washings from the cages, was

immediately digested with the Arnold-Gunning mixture (11), suitably modified to accommodate the somewhat larger samples (0.3 to 1.0 mgm. nitrogen). The digest was then subjected to a Pregl (12) steam distillation in a micro-Kjeldahl apparatus and the distillate collected under boric acid for determination by the method of Meeker and Wagner (13).

RESULTS. A. *The gonadotropic content of the pituitary glands.* Fourteen groups of animals were subjected to varying degrees of protein re-

TABLE 1

The composition of the diets employed

The numbers of the diets are approximately their protein content in per cent

#0 (nitrogen 0.007%)		#2 (nitrogen 0.27%)	
Cornstarch	550	Cornstarch	713
Rice cellulose*	100	Carrot powder§	157
Sucrose	250	Rice cellulose	120
Corn oil	50	NaCl	3
Salt†	50	Na_2HPO_4	7
Fed in 100 gram portions daily mixed with 50 cc. of water containing 5 cc. of tiki tiki‡		Fed in 100 gram portions mixed with 100 cc. of water	
#5 (nitrogen 0.79%)		#16 (nitrogen 2.56%)	
Whole wheat flour	400	Whole wheat	540
Cornstarch	520	Alfalfa leaf meal	400
Alfalfa leaf meal	10	Soy bean oil meal	30
Salt†	30	$CaCO_3$	20
Rice cellulose	40	NaCl	10
Fed in 100 gram portions mixed with 100 cc. of water containing 1 cc. of tiki tiki‡		Fed in 200 gram portions mixed with 300 cc. of water, or fed dry in pellet form	

* Generously supplied by H. J. Heinz Co., Pittsburgh, Pa., to whom we gratefully acknowledge this aid.

† Cf. reference 18.

‡ Harris Laboratories, Tuckahoe, N. Y.

§ Purchased from California Concentrates Co.

striction and sacrificed on the eighth day of pseudopregnancy. The diets employed and the periods of dietary treatment (which include the eight days of pseudopregnancy) were as follows:

9 groups—diet 2 for 14 to 22 days
1 group—diet 0 for 23 days
1 group—diet 5 for 32 days
1 group—diet 5 for 11 days; then diet 2 for 21 days
1 group—diet 2 for 11 days; then diet 0 for 8 days
1 group—diet 2 for 21 days; then diet 0 for 21 days

At autopsy these animals presented normal, fully developed corpora lutea of pseudopregnancy and the average number of corpora lutea per animal was no different from the normal (10.5 ± 2.5). Regardless of the degree or duration of protein restriction the gonadotropic content of the pituitary glands obtained from these animals was entirely comparable to that of control glands taken from stock animals at the 8th day of pseudopregnancy at the same time of the year (fig. 1). It is true that most of the values for the experimental groups fall somewhat below the control line, but in only one instance could this difference be considered significant. This one low value was obtained from a group of animals subjected to diet 2 for 22 days and autopsied in March 1937. Repetition of this experiment

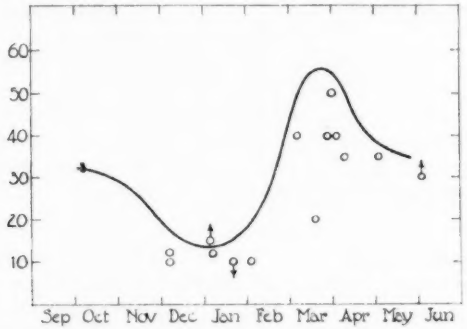

Fig. 1. The solid line represents the seasonal change in gonadotropic content of rabbit pituitaries at the 8th day of pseudopregnancy as determined by assays on the glands of control animals over a two year period. The solid points indicate the assays of glands from the animals in these experiments after variable periods of diet restriction as described in the text. Points with arrows indicate assays which were not definitive, but which yielded values "greater than" (arrow up) or "less than" (arrow down) the solid point from which the arrow arises.

in May of the same year resulted in a gonadotropic content more nearly like that of the control animals of the same month.

Of the 113 post-partum or post-pseudopregnant rabbits which were subjected to the final mating trials 31 refused service. Assays on two groups of glands obtained from nine of these anestrous females revealed a gonadotropic content fully as high as that in the normal estrous rabbit at the same time of the year.

B. *The nutritional state of the experimental animals.* In view of the nutritional state of the rabbits it is surprising that the incidence of anestrus (24 per cent) was no higher than that actually found. In the rat a reduction of 30 per cent in food intake on a complete ration leads to serious abnormalities in the estrous cycle even though body weight is maintained

(4). In our rabbits the transfer from the stock ration to the experimental diets led to a reduction in food intake greatly exceeding 30 per cent (from about 140 grams, or 390 calories, on the stock ration to about 60 grams daily on diet 2 and 55 grams on diet 0, representing about 185 and 165 calories respectively). As may be seen from the example in table 2 body weight was not maintained. Yet there was no correlation between the weight loss of the individual animals and their mating behavior. Indeed, the average loss of the 31 anestrous females was precisely the same as that in the animals which accepted service.

Analyses of liver glycogen and fat, kindly performed by Doctors Harry Vars and Samuel Goldschmidt, allow a comparison of the effects of our experimental diets with those of complete starvation (table 2). It is obvious that the nutritional state of our experimental rabbits was not one

TABLE 2

Glycogen and fat content of rabbit livers at the 8th day of pseudo-pregnancy

DIET	NUMBER OF ANIMALS	WEIGHT LOSS	LIVER GLYCOGEN	LIVER FAT
		per cent		
Stock (#16)	11		5.16	12.28
3 days complete starvation	6	8.5	1.38	14.65
#0—23 days	10	13.0	7.43	24.61
#2—21 days, then #0—21 days	7	20.0	5.98	32.06*

Weight loss is expressed as per cent of original body weight. Liver glycogen is expressed as per cent of wet weight. Liver fat values are total lipids in per cent of dry weight.

* Average of 6 specimens, the 7th being lost.

of simple inanition. On the contrary, the pronounced changes induced in the liver are characteristic of protein starvation (14).

C. *The course of nitrogen excretion.* To determine the rate of protein depletion in our experimental animals it was necessary to follow the course of urinary nitrogen excretion. This was done on nine animals of a group subjected to diet 2 for 21 days and then diet 0 for 21 days. On five of these animals the 24 hour urinary nitrogen excretion was determined at intervals of two to five days starting on the 4th day of the experiment, and on four other animals at irregular intervals starting on the 10th to 15th days of the experiment. As a result we have data on nitrogen excretion of five animals from the 4th to 15th days of the experimental period and on nine animals from the 15th to the 35th days. Due to the variable pattern of excretion from animal it is impossible to present the results satisfactorily by any composite curve. It may be noted, however, that in the five animals investigated most frequently the nitrogen excretion fell to its lowest point between the 6th and 17th days of the experiment (table 3),

and shortly thereafter (2 to 9 days) began to rise. In each of the nine rabbits the nitrogen excretion continued to rise until the end of the experiment despite the change to the protein-free diet on the 21st day, the rise being more pronounced in the rabbits showing the more rapid weight loss. Between the 21st and 35th days of the experiment the urinary nitrogen excretion per 24 hours ranged from 300 to 800 mgm. per rabbit.

DISCUSSION. From our results it appears that the reproductive system of the rabbit is much more resistant to protein deprivation than that of the rat. On a 4 per cent protein diet a majority of rats show complete anestrus in four to eight days, and this is just about the time required (4 days) to reduce the nitrogen metabolism of the rat to the endogenous level (15, 16).[1] From our own data on nitrogen excretion, and from the data of Smuts (17) a comparable period of protein starvation in the rabbit would be about 15

TABLE 3

The time required to reach the lowest level of nitrogen excretion on diet 2

Body weight is given in kilograms. The values listed under mgm./cal. are based upon the basal metabolic rates of the rabbits as calculated from the body weight (19).

RABBIT	WEIGHT	DAY	NITROGEN EXCRETION	
			mgm./day	*mgm./cal.*
1	3.11	17	187	1.15
2	3.01	14	140	0.89
3	3.30	14	184	1.10
4	3.33	10	157	0.93
5*	4.78	6	303	1.39

* Because of its size this rabbit was offered 200 grams of food daily instead of the usual 100 grams, and its daily food intake averaged about twice as much as that of the other animals.

days. In view of the very low protein content of our experimental diets it is safe to say that even in the shorter experiments the rabbits were subjected to a degree of protein starvation entirely comparable to that which produced anestrus in the rat.

While it is possible that the dietary anestrus in the rat may eventually be shown to be due to pituitary hypofunction, similar dietary conditions in the rabbit do not significantly interfere with the gonadotropic function of the pituitary. It is striking that in our experimental animals the rise of the gonadotropic hormone to normal levels occurred after the animals had been reduced to the endogenous level of nitrogen metabolism and were ingesting less nitrogen than they were excreting. Of greater importance is the fact that such restitution occurred at a normal rate in animals on a

[1] The data of Ashworth are in agreement with those of Mitchell and Carman, although his conclusions are not entirely so.

protein free diet in which the only protein nitrogen available was that derived from the tissues.

SUMMARY

1. In the discharged rabbit pituitary the process of gonadotropic hormone restitution, as measured by our methods, was not influenced by protein restriction at least as severe as that which produces dietary anestrus in the rat.

2. Even on a protein free diet the excess of hormone stored during the first eight days of pseudopregnancy, over and above the amount released to the peripheral tissues, was fully equal to the excess stored by the control animals (16 per cent protein diet) in the same period of time.

3. Despite the adverse dietary conditions those factors responsible for the seasonal changes in the pituitary were able to exert their full influence (fig. 1).

4. Consequently we are obliged to conclude that the rabbit pituitary is more responsive to seasonal influences than to changes in dietary protein. Indeed it appears that the protein requirements for the formation of the gonadotropic hormone can be fully met by the nitrogenous products derived from the rabbit's own tissues even during the stress of serious nitrogen deficit.

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VENTRICULAR FIBRILLATION DUE TO SINGLE, LOCALIZED INDUCTION AND CONDENSER SHOCKS APPLIED DURING THE VULNERABLE PHASE OF VENTRICULAR SYSTOLE[1]

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It is generally believed that in order to induce ventricular fibrillation by electric currents they must not only have a minimal intensity but must act for a fair interval of time (cā, several seconds). Indeed, the variation in duration of a current has been used as a criterion of the sensitivity of the heart (1).

In 1934 King (2) and in 1936, Ferris, King, Spence and Williams (3) reported that shocks as short as 0.03 second are effective in fibrillating the ventricles provided they are applied during the occurrence of the T wave, which they interpreted as the partial refractory phase. Previous to this, de Boer (4) had shown that a process similar to fibrillation in mammalian hearts can be induced in the frog's ventricle by induction shocks applied near the end of the systole, but he believed only during a hypodynamic state. Andrus, Carter and Wheeler (5) found that an induction shock similarly introduced into normal auricles of dogs caused auricular fibrillation. During 1923–24 the senior author (6) in studying the response of the dog's ventricles to strong induction shocks demonstrated that the mammalian ventricle is not refractory to stimuli for a considerable, though apparently variable, interval of systole (last 0.03–0.09 sec. of systole). In order to produce premature contractions by shocks applied during systole it was necessary to utilize very strong break shocks, and in the zeal to elicit such contractions ventricular fibrillation all too often terminated the experiment.

A survey of many records has shown that this was due to single shocks and that all were delivered somewhere during the non-refractory phase of ventricular systole. In view of the importance of observations that a very brief shock is capable of inducing fibrillation even when the exciting current traverses only a small area of the ventricle, it seemed important

[1] This investigation was supported by a Grant from the John and Mary R. Markle Foundation.

[2] Fellow of the C.R.B. Educational Foundation.

to repeat these observations more methodically, by using condenser shocks of exceedingly brief duration.

METHODS. Dogs were lightly anesthetized with morphine and sodium barbital, the chest was opened, the heart suspended in a pericardial cradle and intraventricular pressure was recorded by a Wiggers optical manometer.

In order to introduce shocks at appropriately timed portions of late systole, a condenser discharge was released by the aid of a photocell and time delay circuit.

By focussing a single slit lamp (filament lamp of constant brilliancy) on the manometer membrane, a long narrow beam was reflected back to the photokymograph,[3] about $1\frac{1}{2}$ meter away. Another part of the beam moved past the slit of a housing surrounding the photoelectric cell. The flow of current from cathode to anode occasioned when the photocell was momentarily illuminated was properly amplified and activated by a thyratron trigger tube. The voltage thus developed was impressed on a resister-condenser which operated as a variable delay circuit. This circuit was designed so that its critical voltage developed after a predetermined time interval and then overpowered the negative bias on a thyratron-condenser. A high resistance in parallel with the condenser made the thyratron tube a self-extinguishing circuit. When the bias voltage was reduced sufficiently on the discharge tube, it conducted and discharged a 4-micro-farad condenser charged with 300 volts. Any fraction of the voltage discharged through a voltage divider could be used as the stimulus. For stimulation two stigmatic $Ag \cdot AgCl_2$ electrodes were directly applied to the heart. The distance between the electrodes was about 1 cm.

The time of the condenser-discharge was recorded by shunting a small fraction of the discharge to a General Electric oscillograph, recording on the photokymograph with the pressure curve.

During each stimulation the quantity of current used for stimulation was determined by a ballistic galvanometer previously calibrated with an external shunt of 2000 ohms in circuit. Since the effective resistance of the heart could not be determined each time, these values are approximate only, but serve as relative values if it be assumed the heart resistance did not change.

In order to repeat observations on the fibrillating effectiveness of shocks on dogs, the ventricles of which do not recover spontaneously, provision for prompt revival by countershock needed to be made. The procedure of Hooker, Kouwenhoven and Langworthy (7) was adopted. To assure prompt revival without manipulation, massage, or use of drugs—all of which might alter irritability, two large metal electrodes were kept permanently in place, one on each lateral aspect of the ventricles. By throw-

[3] The optical system utilizing double slit lamps or the projection system for Hamilton or Gregg manometers does not lend itself to such projection.

ing two switches in quick succession, the stimulating apparatus was protected and the countershock was applied. Thus, revival was achieved in 15 seconds to 1 minute.

The only unfortunate feature of the arrangement was that the presence of large metal electrodes deformed standard e.c.g. leads; indeed, this necessary feature for revival prevented use of the R wave to set off the stimulus, a method so effectively used by others.

Results. Repeated tests on seventeen different dogs demonstrated consistently that stimuli falling during the rise of intraventricular pressure are without effect, that those applied during the summit at various intervals up to about 0.06 second before the end of contraction, marked by the incisura, produce either a small premature contraction early in following diastole, or ventricular fibrillation, and that those given during isometric relaxation or subsequent diastole cause premature contractions only. It may be added that strong break induction shocks generated by a Harvard induction with core removed again yield similar results.

Small sections from a continuous series of observations are reproduced in figure 1, the strength of stimulus being expressed in coulombs. It may be noted that the current variations are not great.

The first curve, A, shows the typical premature contraction starting at *P* resulting from a shock at the beginning of isometric relaxation. Curve B shows that a slightly weaker shock delivered at the summit of the pressure curve, or 0.064 second preceding end of mechanical systole (at *X*), resulted in fibrillation. Close inspection reveals a slight tendency to alternation in this record and the stimulus fell during the smaller beat. Alternation or other hypodynamic states are not a *sine qua non* for induction of fibrillation, as was amply demonstrated on other dogs. If the forms of the first and third beats are carefully compared, we note the same tendency to start a premature contraction at *P* as in the curve of record A. This was followed by several inefficient contractions (P_1, P_2) that only produce slight variations of intraventricular pressure. The heart was promptly revived by countershock and in less than one minute was beating normally. Approximately five minutes later, record C was obtained. A shock administered toward the end of isometric relaxation caused a larger premature contraction than that shown in curve A. Very shortly thereafter a similar shock was delivered to the left ventricle during the peak of systole, or 0.027 second before its termination and fibrillation was again induced. As the experiment continued, the ventricular beats became more hypodynamic. The other records show that fibrillation was consistently produced by shocks during late systole, and it may be added, never by shocks delivered during any other phase of the heart cycle. Fibrillation and recovery were thus produced ten times; the animal did not die in fibrillation. This again emphasizes the efficiency of the method of countershock.

Discussion. The results reported confirm previous investigations in

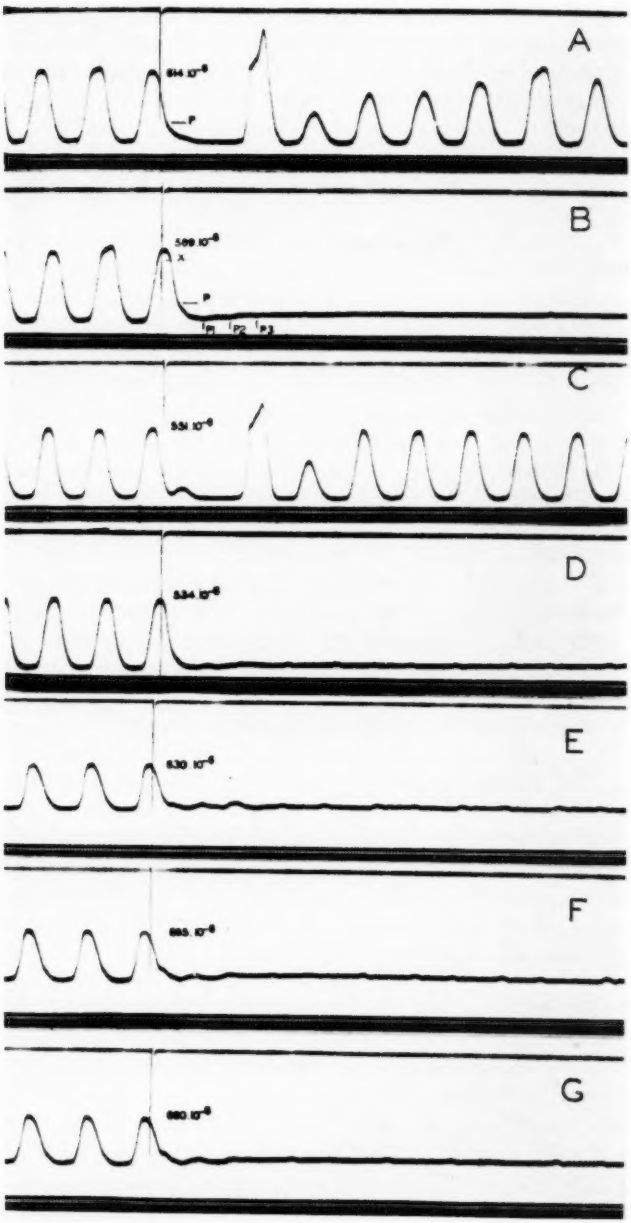

Fig. 1

establishing that a considerable portion of late systole constitutes a *vulnerable period*, during which alone stimuli are effective in inducing ventricular fibrillation. This corresponds roughly to the T wave of a standard electrocardiogram, although in our experience great variability exists in the relation of this wave to the end of systole.

On the basis of our experience, this vulnerability is inherent in normal hearts; it occurs in young dogs as well as old, and in vigorously beating as well as hypodynamic or alternating ventricles. The latter is obviously not a requirement for production of fibrillation, as de Boer (4) has postulated for the frog ventricle.

The demonstration that fibrillation is only induced by a single shock delivered to a localized region of the ventricle when it falls during the vulnerable period makes it obvious that the induction of fibrillation—i.e., the physiological condition necessary for converting coördinated into incoördinated contractions—does not require passage of a current through every fraction of the myocardium, as was the case in experiments reported by the Columbia investigators (2, 3). Hence the probability that fibrillation following drugs or myocardial ischemia may likewise be initiated by localized excitation, and does not necessarily require a generalized altered irritability or reactivity.

If fibrillation is only induced by effective stimuli that fall during the vulnerable period of late systole, some *a priori* conclusions may be drawn as to the manner in which more prolonged currents induce fibrillation. A direct current lasting only two seconds would cover four complete heart cycles at a heart rate of 120/min. In accordance with well established laws of stimulation such currents would most probably induce fibrillation if either the moment of opening or closing fell during this period. In the case of an alternating current, say 60 cycles, the closing of the circuit during a vulnerable period would likewise constitute a particularly dangerous event since this represents its highest stimulating value. However, effective changes in intensity still occur several times during each vulnerable period, any one of which could serve as *the* effective stimulus inducing fibrillation (8). This offers an explanation for the generally recognized fact that alternating currents are more dangerous than direct currents at lower ranges of effectiveness, for the chance of a direct current being opened or closed during the brief vulnerable period is not great. However, unless passage of alternating current during one cycle alters irritability for the next it may be anticipated that fibrillation is always induced during the first vulnerable period that it strikes.

The corollary follows that no *a priori* reason exists for supposing that the duration of application of any current bears any relation to its fibrillating efficiency, or that it can be used as a criterion either of the sensitivity of the ventricles to fibrillation or in estmating the value of agents supposed to increase or decrease such sensitivity.

It may be admitted that such conclusions fail to take into account the possible effects that passage of currents during a series of beats have on irritability, but in such event some fortuitous stimulus must operate during some vulnerable period to start the fibrillation. Many possibilities may be thought of, such as pressure changes within the heart, mechanical stimuli with movements of the heart, chance variations in current strength passing through the myocardium during the vulnerable period, etc. But if fortuitous stimuli act to induce fibrillation when currents are applied for various durations of time, the variability of current duration inducing fibrillation is not a scientific criterion of ventricular sensitivity to stimulation, because the intensity of the fortuitous stimulus and time of its action are unknown and probably variable.

SUMMARY

Experimental evidence is reported which demonstrates that a brief induction or condenser shock applied to normal hearts of old or young dogs by stigmatic electrodes induces fibrillation only when the shocks fall during the vulnerable period of late systole. Since the effect is obtained by use of stigmatic electrodes, passage of the current through the whole or large parts of the myocardium is not necessary to initiate fibrillation.

Several implications are discussed: 1. Fibrillation following use of prolonged electrical currents, drugs, chemicals and ischemia may likewise be induced by some effective stimulus—possibly a fortuitous one—falling during the vulnerable period. 2. One reason why an alternating current at critical voltages is more dangerous than the direct current seems to lie in the fact that effective variations of current strength obviously fall during the vulnerable phase in the former and only during closing and opening of the direct current. 3. The experimental results indicate that the effectiveness of currents or the variations of myocardial sensitivity to fibrillation cannot be tested experimentally by noting variations in the duration of current flow, a criterion frequently employed by previous investigators.

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EVIDENCE OF PITUITARY INVOLVEMENT IN THE EXPERIMENTAL CONTROL OF WATER DIURESIS[1]

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Stimulation of the lumbar region in the bitch is reported to inhibit water diuresis for appreciable periods (1, 2), a response which denervation of the kidney fails to alter. Verney (3) has ascribed this response to release of antidiuretic principle through reflex stimulation of the posterior lobe of the pituitary body. This concept was advanced as a more reasonable assumption than the contingence of nonspecific factors acting directly to influence the internal partition of water or to induce effective vasomotor changes (e.g., 4, 5), but it was pointed out (2) that ultimate "proof or disproof of the involvement demands and depends upon experiments of a more direct nature."

A decisive test of the presumptive reflex release of antidiuretic hormone would consist in lumbar stimulation after interruption of the hypothalamo-hypophyseal connections since, if Verney's view is correct, this should forestall the inhibition of diuresis characteristic of the intact animal. The present report is concerned with observations, derived by this means, which it is believed yield evidence of pituitary participation in the response to certain afferent stimuli. This type of attack was made feasible through use of a stereotaxic apparatus whereby it was possible to approach the hypophysis intracranially (6).

The experiments fall into two groups, in the first of which the rôle of the hypophyseal complex in the antidiuretic response to lumbar stimulation is considered; in the second series are included attempts to inhibit water diuresis by stimulation directly of the pituitary stalk. A brief report of the first phase of this work has been given elsewhere (7).

METHODS. The problem of a suitable experimental preparation presented difficulty at the outset, particularly since attempts at the use of unanesthetized animals were unsatisfactory. Anesthetics exert a notoriously depressant effect upon water diuresis, but, of a number tried, chlora-

[1] This investigation was supported in part by a grant from the American Academy of Arts and Sciences. The work was begun in the summer of 1938 at the Biological Laboratory, Cold Spring Harbor, where facilities were generously provided.

lose-urethane proved least objectionable in this respect, so long as one dose sufficed. When the necessity for prolonged deep narcosis made additional doses imperative, however, difficulty was met in maintaining an effective urine flow, so that diuresis was by no means an invariable sequel of hydration, and many animals were discarded through failure to develop an adequate polyuria. Diuresis, when it did occur, however, usually reached a high level. For our purposes rather arbitrary standards were set: no animal was considered unless the hourly urine output exceeded by five times the normal rate for that animal as previously determined; inhibition was considered significant if the rate of flow was reduced by 50 per cent or more and if it persisted at a reduced level for at least one hour; finally, if the rate of flow failed to show a subsequent rise, the experiment was rejected.

Adult, well nourished rabbits of both sexes were used. In a typical experiment, a rabbit was placed on a lettuce and water ration in the afternoon, following a determination of hourly urine output.[2] The following morning the animal was anesthetized (2 per cent chloralose in 10 per cent urethane, 5 cc. per kgm., intravenously) and given tepid water, 4 per cent of its body weight, by stomach tube. The bladder was catheterized, and its contents were expressed and recorded at 30-minute intervals. Three to four hours later, when the initial diuresis had largely subsided, a second dose of water was given. The resultant diuresis usually began within 30 minutes, the curve rising rapidly; during its rise the appropriate procedure was undertaken.[3]

Control animals, prepared two to four days in advance, were subjected to interruption of the pituitary stalk by electrolytic lesion. Under Nembutal (pentobarbital sodium) anesthesia the skull was trephined aseptically at bregma and oriented in a stereotaxic instrument similar in principle to one employed in previous work (6, 8). Electrodes, of 22-gauge Nichrome wire, insulated to the tips, were placed in the stalk and a burn was produced by direct current of 0.3 to 0.5 milliampere strength at two levels, separated 1 mm., in the stalk and gland. To ensure adequate destruction burning was continued for 3 to 5 minutes at each level.

In experiments involving stimulation of the stalk, the anesthetic and the initial dose of water were given, following which the skull was trephined and oriented. With appearance of the second diuresis, electrodes were placed in the stalk. In stimulation a Harvard inductorium, driven by two dry cells and with the vibrator weighed to give approximately 6 to 8

[2] Considerable variation in normal output was observed, as it ranged from 1½ to 5 cc. per hour, as determined over 5 to 10 hour periods. Expressed as cc./sq.m./min., urine flow varied from 0.18 to 0.54 in the animals included in this report.

[3] I am grateful to Mr. John Gijsbers of this laboratory for assistance in preparation for experimental routines.

shocks per second, supplied the current, at a coil distance variously of 7 to 9 cm. Stimulation was applied immediately after a urine flow determination and continued intermittently for 10 to 15 minutes. To facilitate later identification a direct current burn was placed at the site prior to withdrawal of the electrodes. Controls were prepared precisely as for the first group. At sacrifice the heads were injected with 10 per cent formalin, and after hardening the brain and pituitary were carefully examined. The site of lesion, typically, was indicated by a blood clot, and the gland in the control groups usually appeared hemorrhagic and badly discolored. Facilities were not available for microscopic preparation, a desideratum of prime importance as a definitive check upon the localization of lesions produced.

Investigation of the effects of pituitary stalk stimulation proved considerably more difficult than had been anticipated. In the first place the continuous deep narcosis required militated against establishment of an adequate polyuria. Placing an animal in the stereotaxic apparatus, despite profound anesthesia, invariably caused abrupt diminution in urine flow, and subsequent gavage frequently resulted in death, presumably from water intoxication. Occasionally, however, good diuresis was established; in all, eight experimental and six control animals became available in which it was possible to apply stimulation during the second diuresis, with results which it is felt justify their inclusion in the present report.

Vagotomy was performed in a number of animals. A few failed to respond to hydration and were discarded; others responded fairly well and are included in the appropriate tables. The extreme difficulty in obtaining satisfactory diuresis in the acute preparation following surgical manipulations discouraged further interference; hence the splanchnic nerves were left intact and blood pressure recordings, after some initial attempts, were omitted. Both circumstances would appear legitimate, however, in view of the type and behavior of our controls, and on the basis of observations reported from other laboratories (9, 10).

EXPERIMENTAL RESULTS. *Series I. Afferent stimuli and destruction of the stalk.* It became necessary first to determine whether the rabbit resembles the dog in developing an antidiuretic response to lumbar stimulation. In a control series of six animals, the lumbar region was depilated and, during the rising tide of the second diuresis, a large hypodermic needle was inserted into the tissues between the 4th and 5th lumbar spinous processes and twisted about. Stimulation was continued for ten minutes. Since narcosis, now some five hours after its induction, was becoming lighter, the animals sometimes struggled slightly. In no case did inhibition fail to occur, followed, ultimately, by a rise as the antidiuretic effect of the stimuli subsided—a rise which indicated a preparation still capable of physiological response to the residual water load. The post-stimulation records of three animals are shown in figure 1, in which it is seen that in-

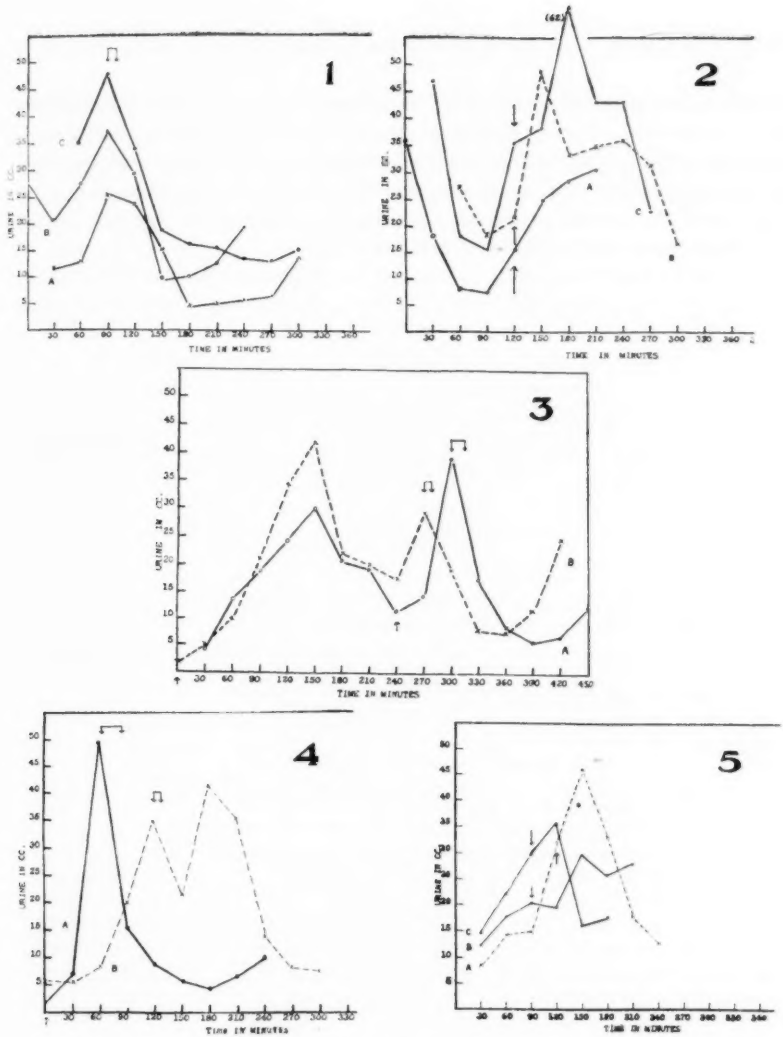

Fig. 1. Lumbar region stimulation, intact animals. *A* — rabbit 2; *B* — rabbit 1; *C* — rabbit 3 (table 1 A). Arrows indicate stimulation.

Fig. 2. Effect of stalk destruction upon response to lumbar irritation. Rabbit 11, records at 7-day intervals. *A* — deep narcosis; *B* — medium narcosis; *C* — light narcosis. See table 1 B.

Fig. 3. Stalk stimulation and water diuresis. Showing initial diuresis, hydration and second diuresis, and (arrows) stimulation of stalk. *A* — rabbit 1, *B* — rabbit 4 (table 2 A).

Fig. 4. Stalk stimulation and water diuresis. *A* — rabbit 3, showing antidiuretic response. *B* — rabbit 6: transitory depression with rapid recovery. (Cf. table 2 A.)

Fig. 5. Stalk destruction and response to infundibular stimulation. *A* — rabbit 9; *B* — rabbit 10; *C* — rabbit 11 (table 2 B). Arrows indicate point of stimulation.

hibition may persist for so long as 180 minutes. It was evident, therefore, that rabbits will react to lumbar irritation by definite signs of antidiuretic activity. Results in this and in succeeding experiments are incorporated in tables 1 and 2, in which urine flow is expressed in terms of cc./sq.m. S.A./min., the surface area in each instance calculated by use of the constant employed by Kaplan and Smith (11).

Following this series, nine animals were prepared by electrolytic interruption of the infundibular stalks. Two to four days later, depending

TABLE 1

Effect of afferent stimuli upon water diuresis

ANIMAL NUMBER	WEIGHT	SURFACE AREA	URINE FLOW IN CC./SQ.M.S.A./MIN. COLUMNS i AND ii—RATE OF FLOW DURING HALF-HOUR PERIODS IMMEDIATELY PRECEDING STIMULATION. COLUMNS iii, iv, ETC.—SUCCESSIVE 30-MINUTE INTERVAL RATES FOLLOWING STIMULATION										
			(i)	(ii)		(iii)	(iv)	(v)	(vi)	(vii)			
A. Intact animals													
	kgm.	*sq.m.*											
1	3.85	0.291	3.26	5.61		3.29	1.41	1.50	1.88	2.88			
2	2.71	0.173	4.01	4.42		2.89	0.87	0.96	1.16				
3	3.35	0.199	6.80	8.90		5.65	3.00	2.65	2.58	2.16	2.05	2.50	Vagotomized
4	2.50	0.164	7.37	10.49		7.37	5.04	3.35	3.12	2.90	2.68	2.74	3.90
5	2.80	0.177	2.19	3.76		2.28	1.88	3.02	3.99	4.74			
6	2.71	0.173	2.31	5.29	Stimulation	4.97	2.67	0.58	0.75	1.88			Vagotomized
B. Controls: pituitary stalks destroyed													
7	3.50	0.205	2.59	5.74		6.13	10.03	6.96	6.56	3.70			
8	3.25	0.197	1.59	3.89		8.57	5.86	6.18	6.44	5.59	3.01		
9	2.50	0.164	1.50	3.09		4.99	5.70	6.18					
10	2.95	0.182	1.79	4.34		6.23	7.09	4.02	2.73				Vagotomized
11	2.70	0.172	1.45	2.99		4.71	5.39	7.54					Jan. 4, 1939
11	2.84	0.178	3.36	4.09		9.13	6.22	6.49	5.88	5.93	3.14		Jan. 11, 1939
11	3.00	0.185	2.79	6.17		6.80	11.12	7.72	7.72	4.10			Jan. 18, 1939
12	2.75	0.174	2.09	5.61	Stimulation	6.08	5.46	2.70	1.43				Stalk intact*

* Lesion in lateral hypothalamus.

upon their good recovery, urinary output was determined, following which the same routine was instituted as in the preceding group. Six of these animals were available for stimulation during diuresis and, with one exception (no. 12, table 1 B) in which the stalk subsequently was found intact, persistent and at times drastic irritation in the lumbar region failed to evoke a characteristic antidiuretic response. In one instance a transitory lag in the rising curve appeared (no. 11, table 1 B). Stimulation was prolonged to 15 and in one case 21 minutes, with no definitely depressing effect. The procedure was carried out three times, at seven-day intervals,

on one animal (no. 11, fig. 2 and table 1 B), both under light and under deep anesthesia, with the same negative result.

Series II. Inhibition of diuresis by stimulation of the pituitary stalk. Figures 3 and 4, and table 2 A epitomize results obtained in the experimental group of this series. Of eight animals, five showed definite inhibition of urine flow; in three instances (nos. 6, 7, 8) transitory depression appeared during the period of stimulation. In one of these (no. 7) *post*

TABLE 2

Effect of pituitary stalk stimulation upon water diuresis

ANIMAL NUMBER	WEIGHT	SURFACE AREA	URINE FLOW IN CC./SQ.M.S.A./MIN. COLUMNS i AND ii—RATE OF FLOW DURING HALF-HOUR PERIODS IMMEDIATELY PRECEDING STIMULATION. COLUMNS iii, iv, ETC.—SUCCESSIVE 30-MINUTE INTERVAL RATES FOLLOWING STIMULATION								
			(i)	(ii)		(iii)	(iv)	(v)	(vi)	(vii)	
A. Intact animals											
	kgm.	*sq.m.*									
1	3.00	0.185	2.60	6.92		3.14	1.43	1.08	1.25	2.24	
2	2.27	0.154	3.78	6.27		4.00	1.72	1.62	2.60	5.41	
3	3.30	0.198	1.17	8.23		2.52	1.51	1.01	0.75	1.09	
4	2.30	0.155	3.74	6.23		4.06	1.71	1.61	2.58	5.35	
5	3.86	0.219	4.56	5.60		1.82	0.55	2.28			
6	3.00	0.185	1.06	6.26		3.86	7.45	6.26	2.51	1.43	Brain not examined
7	3.00	0.185	1.89	7.34	Stimulation	5.72	6.46	6.32	1.08	0.89	Stimulation point 1–2 mm. lateral to base of stalk
8	2.27	0.154	3.47	4.24		4.01	6.91	4.62	1.60		Stimulation point centered in stalk
B. Controls: pituitary stalks destroyed											
9	2.72	0.174	2.90	6.27		9.00	6.44	3.42	2.45		
10	3.80	0.216	3.45	4.60		5.52	2.52	2.76			
11	2.42	0.160	3.71	4.26	Stimulation	4.05	6.12	5.50	5.83		
12	2.80	0.177	6.60	7.28		1.48	0.99	2.54			Stalk intact; lesion in ant. lobe
13	3.20	0.193	2.76	3.32		2.00	1.52	1.22	2.17		Stalk intact; lesion in ant. lobe
14	2.60	0.168	1.15	3.41		3.11	5.05	2.91	1.97	0.21	

mortem examination revealed the point of stimulation between 1 and 2 mm. lateral to the base of the stalk. In no. 8 the electrodes apparently had been perfectly placed and beyond the possibility of mechanical injury, an explanation of the failure in response is not available. The brain of no. 6 was accidentally discarded before examination.

Six control animals, in which the stalk had presumably been interrupted, differed markedly in their responses to similar stimulation (table 2, B).

In four instances little or no inhibition occurred (nos. 9, 10, 11, 14), although in two of these (nos. 11, 14) a slight fall ensued during the succeeding half hour, followed by a rapid rise (fig. 5, *B*). In the remaining two rabbits, diuresis was promptly checked. Autopsy revealed intact stalks, the burn in each case situated deep in the anterior lobe.

Discussion. From this work two conclusions emerge which signalize the rôle of the hypophysis in the experimental control of water diuresis. First, since destruction of the stalk blocked the response, inhibition produced by the afferent stimuli in intact animals must have involved participation of the hypophyseal complex. This circumstance would appear to provide direct substantiation of Verney's postulate, so far, at least, as the antidiuretic *modus operandi* of lumbar irritation is concerned. Secondly, interruption of the pituitary stalk will block the antidiuretic response to electrical stimulation in the anesthetized rabbit, at least under the experimental conditions employed.[4]

It seems scarcely necessary to point out that the hypophyseal complex is not the only factor responsible for reduction of urinary flow from diuretic levels, since it appears likely that certain bodily conditions may affect adversely the course of polyuria through as yet unknown but presumably non-specific channels. The primary obstacles to unqualified acceptance of Verney's hypothesis were emphasized originally in the observations of Fee (12) and of Newton and Smirk (13), made upon decerebrate-hypophysectomized preparations. Even taking into account a presumptive release of antidiuretic substance during operation and the possible complication of shock (3), the low level at which urine flow remained for some hours after pituitary removal, and the persistent lag in successive responses to water administration indicated that the pituitary is not wholly indispensable in certain phases of diuresis regulation. These observations and their bearing upon the theory are fully discussed elsewhere (3, 13). Additional evidence against the rôle of the pituitary as sole arbiter of water exchange may perhaps be adduced from incidental observations made by Ingram (14) on cats displaying the chronic polyuria of experimental diabetes insipidus. In such animals marked reduction in urine output and in water consumption followed upon unpleasant handling (e.g., repeated catheterization or gavage), under conditions which would seem definitely to preclude pituitary involvement. In some instances, however, food restriction introduced a complicating factor. In our experience excessive struggling or profound narcosis prevented diuresis in animals with pituitary

[4] Antidiuretic responses to electrical stimulation in the hypothalamus of the cat have been observed by Doctor Ingram (personal communication) who very kindly has placed his results at my disposal. In a limited series intracranial stimulation in and adjacent to the supraoptic fibers elicited a prompt response; similar stimulation in the lateral hypothalamus proved ineffective.

stalks destroyed, but in view of the short-time intervals involved the possibility of escape of residual antidiuretic substance should not be overlooked. For obvious reasons the use of unanesthetized and trained animals is highly desirable—a combination difficult of realization, however, in the use of rabbits and under the requirements which the present experiments impose.

SUMMARY

Water ingestion in rabbits anesthetized with chloralose-urethane provoked a marked diuresis which was curtailed by painful stimulation in the lumbar region, an observation which confirms that of Theobald for the dog. In a series of animals with pituitary stalks destroyed, however, no antidiuretic response occurred upon similar stimulation.

In intact rabbits, anesthetized and hydrated, electrical stimulation of the pituitary stalk resulted in marked diminution in urine flow, a reduction which persisted for appreciable periods following withdrawal of stimulation. Control animals, in which the pituitary stalks had been destroyed prior to stimulation, showed no significant diminution in rate of urine flow.

These observations support the concept of the hormonal nature of the pituitary antidiuretic principle and verify the hypothesis that, under certain conditions at least, its release is under reflex control.

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THE CORRELATION OF ACTIVITY AND TRANSPORTATION IN THE COLON OF THE DOG

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The movement of material through the colon may be dependent upon the strength of activity and the duration of active periods or upon certain specific types of activity. The strength of activity in different segments of the colon may vary greatly from minute to minute. At different times the duration of active periods is also a variable even in the same animal (1). The kinds of activity occurring in the colon have been described as types I, II and III, any one of which may appear simultaneously upon several segments of the colon or in succession in such a manner as to give peristaltic or anti-peristaltic effects (2).

The periodicity of the colon relative to the active and quiet periods has been discussed in considerable detail and is considered by some authors as an essential quality (3, 4). This periodicity is usually apparent in a given segment independent of the activity in other segments during a given time. With the tandem balloon system recording from six segments simultaneously, activity is usually present in one or more of the segments at all times even though during a 400 minute experiment the essential periodicity can be seen in each segment. The importance of these periods in propelling material through the colon has been one largely of presumption rather than knowledge obtained through data. Templeton and Lawson (2) referred to an active period in the "distinctly periodic proximal colon" as consisting usually of type I and type II contractions accompanied by tone changes (type III contractions) of considerable amplitude. During the first part of a period the beginning of activity was usually progressively delayed, distalward from the cecum, in appearance on adjacent segments. Toward the end of an active period the activity frequently began and ended simultaneously in adjacent segments or was delayed oralward.

While the tandem balloon type of apparatus which we have used in previous studies has yielded considerable information relative to the character of colon motility, no satisfactory correlation could be made between activity and transportation. Also inadequate for such correlation is the

sponge rubber bolus technique used by Quigley, Highstone and Ivy (5) who presented evidence that the motor activity of the gastro-intestinal tract could be divided into movements which mix materials without appreciable alteration of position of those materials, and movements which transport materials.

In order to make a correlation between activity and transportation it was necessary that an apparatus be devised which when inserted by way of a cecostomy would be free to move and at the same time transmit pressure changes indicative of activity. This apparatus (6) consisted of a balloon which, when not inflated, measured 10 cm. in length and 4 cm. at its widest diameter, attached to a small rubber tube $\frac{3}{32}$ inch bore. The tube graduated in centimeters was attached to a water manometer by way of a T tube.

The experimental data were obtained from five dogs which had previously been cecostomized and trained to lie quietly for several hours at a time on a cushioned table. The balloon of the recording apparatus was inserted into the colon, by way of a cecostomy, to a depth of 5 cm. beneath the skin, and alternately inflated and deflated several times to smooth its contour. Six centimeters of negative water pressure were then sucked against the balloon for standard deflation. With the deflated balloon clamped out of the system, the manometer pressure was raised to 8 cm. of water above atmospheric pressure. Continuity between the balloon and manometer was then established and thus a closed system for recording obtained. Throughout the course of each experiment the graduated tube which was free to move was observed continuously. Each centimeter of tube movement was recorded with a signal magnet at the time it occurred and correlated with the motility indicated at that instant.

The tracings thus obtained in the 200 minute experiments were first divided into four 50 minute periods, and a general correlation made between the percentage of activity and the centimeters of balloon transportation. The quantity of activity was determined by measuring the duration of activity in terms of minutes. Activity was considered to be present if the duration was one minute or more, and a quiet period existed if no activity appeared in the course of 2 minutes. The data thus obtained (table 1) revealed activity to be present about 50 per cent of the time during each of the four 50 minute periods. On the other hand, transportation of the balloon was found to be greatest in the first 50 minutes and gradually decreased until the close of the experiment. The factors which may be concerned in this diminution in transportation efficiency may be related to: 1, initial irritation brought about by insertion of the balloon, and 2, to an *efficiency gradient* in the colon. That this efficiency gradient is not entirely dependent upon the percentage of activity as calculated by time is shown in our data which revealed the duration of activity in the

fourth 50 minute period to be as great as in the first 50 minute period. If the greater transportation in the first 50 minute period was due simply to initial stimulation of activity by irritation caused by balloon insertion, then quality of activity must have been affected rather than quantity. Experiments, conducted with stationary balloons, however, revealed that the quality of activity at the end of a 200 minute experiment was indistinguishable from the activity at the start of the experiment. These facts seem to belittle the importance of initial irritation, caused by the balloon when properly inserted, as being responsible for the greatest amount of transportation occurring in the first period of the experiment.

The great majority of interfering factors which might conceivably alter colon activity are controllable. Unanesthetized, properly trained animals are not influenced by external interferences, such as noises, usually encountered during an experiment. Moderate deviations from a standard distention of the proximal colon of the dog such as we have used have been reported by Lawson and Templeton (7) to be followed by only tem-

TABLE 1

Average number of minutes of activity and centimeters of transportation per 50 minute period in proximal colon

	50 MINUTE PERIODS			
	1	2	3	4
Minutes of activity	26	29	27	27
Centimeters of transportation	5.3	4.5	3.6	2.6

porary augmentation of proximal colon tone activity. The time of feeding prior to an experiment and the type of food discussed by Templeton and Borkon (8) was followed in this work. The position of the animal during an experiment was practically the same from day to day, namely, lying comfortably on the left side. The experiments which are reported in this paper were conducted during the fall and winter months, at which time the temperature of the room was maintained for the comfort of the animals. Sleep, as indicated by the type of respirations, snoring and isolated paw movements which were occasionally observed, has been reported (8) to have no significant effect upon the quality or quantity of colon activity.

Further analysis of the data obtained revealed from 3 to 10 active periods in all tracings, varying in duration from 1 to 136 minutes. Approximately 48 per cent of the groups were less than 12 minutes in duration, while 83 per cent were 24 minutes or less.

A detailed study of each tracing relative to tube movement showed that transportation with few exceptions was intimately related to the active period. For a detailed consideration, each active period was divided into

four equal parts by vertical lines (fig. 1) in order to make a definite correlation between transportation and activity. Data thus obtained revealed that during the first and second quarters an average of 1.37 and 0.57 cm.,

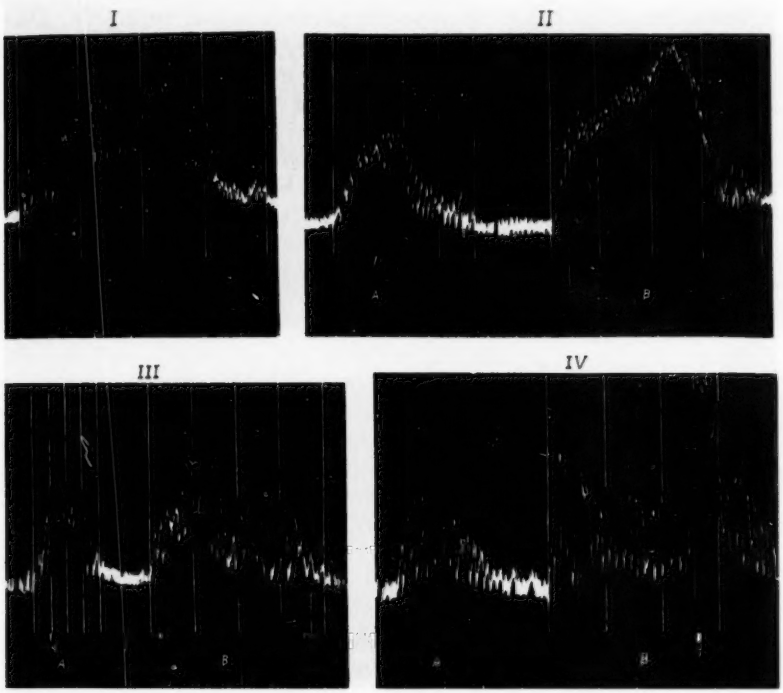

Fig. 1

TABLE 2

Amount of transportation per quarter in 267 active periods

	ACTIVE PERIODS DIVIDED INTO 4 EQUAL PARTS			
	1	2	3	4
Total number of centimeters of transportation	367	153	55	54
Average number of centimeters of transportation	1.37	0.57	0.2	0.2

respectively, of tube movement occurred, while only 0.2 cm. of movement occurred in each of the third and fourth quarters (table 2). Thus it was determined that in the active period an average of 58 per cent of propulsion

occurred in the first quarter and 82 per cent in the first half. The percentage of propulsion of the third and fourth quarters was small but evenly distributed.

Constant observations have indicated that the first contractions of an active period are most important insofar as propulsion is concerned. Tube movement (fig. 1, part 1) may be observed simultaneously with the start of an active period, and fail to appear again in either the third or fourth quarters even though activity may appear to be of considerable strength. Occasionally movement of the tube is observed to precede activity as indicated by the balloon (fig. 1, part 2a). An interpretation of this phenomenon may be that activity started proximal to the balloon and acted directly on the tube or upon material between the balloon and the site of activity causing transportation of the tube. Such an interpretation gains strength from the fact that when such transportation occurred it was usually followed within one or two minutes by activity upon the balloon. Propulsion of this type, however, was relatively infrequent, obviously because very strong transporting movements would be necessary in order to propel a small bore slippery tube, or exert sufficient force through intervening material for an appreciable distance; consequently almost all transportation could be correlated with pressure changes in the balloon.

Active periods of more than average intensity were frequently no more efficient in transporting the tube than periods of much less intensity. Quigley, Highstone and Ivy also noted that increased or maintained high tone in a Thiery-Vella loop might result in stagnation in spite of moderately vigorous peristalsis.

That the intensity of a period, as indicated by pressure changes in the balloon, is not the controlling factor in propulsion has been frequently observed. Periods of relatively low intensity (fig. 1, part 3a) may be very effective in propelling material, while periods of good intensity (fig. 1, part 4a) may be relatively ineffective insofar as transportation is concerned. Active periods in the proximal colon of good intensity, but with no propulsive efficiency, constitute about 22 per cent of the total number of active periods. From these observations it is obvious that differential characteristics which distinguish propulsive types of activity from the non-propulsive types in any given active period cannot be determined with this recording system.

While the majority of active periods present only one good type III contraction or tone change, occasionally two or more tone changes of considerable degree are found (fig. 1, part 4b) in a single active period. In those instances in which two or more type III contractions appear without an intervening quiet period, transportation which seems to be intimately related to the type III contraction was necessarily tabulated as occurring in the third or even fourth quarter of an active period. Con-

sequently a method of tabulation which would consider only type III contractions would give a higher percentage of transportation occurring in the first quarter than the method used which considers only active periods. However, the values previously cited of 58 per cent propulsion in the first quarter and 83 per cent in the first half of an active period seem significant.

In view of the definite correlation which was found to exist between certain parts of an active period on transportation efficiency in the proximal colon, it seemed advisable to make a similar study of the distal colon. For this series the same animals were used, with a technique especially devised. The apparatus consisted of a balloon of the dimensions previously described attached to a graduated rubber tube which was reënforced with a coiled wire spring in the lumen to afford sufficient rigidity to prevent a kinking of the system. The balloon was inserted by way of the anus to a distance of 16 to 18 cm. After smoothing the contour of the balloon it was connected with pressure in the water manometer as previously described. The tube, which was free to move, was watched carefully and each centimeter of movement was recorded by a signal magnet placed immediately below the writing point of the manometer.

TABLE 3

Average number of minutes of activity and centimeters of transportation per 50 minute period in distal colon

	50 MINUTE PERIODS			
	1	2	3	4
Minutes of activity	18	17	18	19
Centimeters of transportation	1.5	1.2	1.0	1.0

For comparison with transportation and activity in the proximal colon, the data obtained in this series were treated in the same manner as those previously described (table 3). The percentage of activity in each 50 minute period of this series was found to be about 34 per cent, as compared to 50 per cent in the data obtained from the proximal colon.

The number of active periods observed varied from 4 to 10 per tracing, while the duration varied from 1 to 152 minutes. These figures are comparable to those obtained on the proximal colon. Approximately 70 per cent of the active groups were less than 12 minutes in duration, while 86 per cent were 24 minutes in duration, or less. Obviously short active groups are more prevalent in the distal colon than in the proximal where only 48 per cent of the active groups were less than 12 minutes in duration. In this segment, however, as was the case in the proximal colon, transportation was intimately associated with the active period. The data

obtained from this distal series showed tube movement in the first and second quarters of the active period to be 0.5 to 0.13 cm. respectively, while that of the third and fourth quarters was 0.07 and 0.06 cm., respectively. From these figures it was determined that during the first quarter of an active period, 66 per cent of the total transportation occurred, and 84 per cent during the first half, which is also comparable to the data obtained from the proximal colon. In the distal, as in the proximal colon, the strength of activity cannot be used as an index to transportation efficiency. In contrast to proximal colon, where it was shown that 78 per cent of the active groups did some transportation, only 50 per cent of the active periods in distal colon were effective in that capacity.

SUMMARY

1. A technique is described by which propulsion of a bolus through a segment may be correlated with the activity of that segment.

2. The essential periodicity of colon activity as described by others is confirmed with this technique.

3. Propulsion is found to be intimately associated with the active period in both proximal and distal colon.

4. The first quarter of an active period is most efficient in transportation in both segments of the colon.

5. Studies on the proximal colon show that the rate of transport of the bolus is greatest when it is in the most proximal segments.

6. Studies of the distal colon reveal that the rate of propulsion of a bolus is also greatest when it is in a less distal segment. However, compared with proximal colon the rate is less.

7. Studies of the colon reveal that transportation efficiency of an active period is greatest in the most proximal segments.

The authors are indebted to Dr. A. J. Carlson, who made this study possible.

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THE EFFECTS OF GROWTH AND ATROPHY UPON THE STRENGTH OF SKELETAL MUSCLE

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In these experiments, concerned with the effects of growth and atrophy upon the strength of the rats' intact gastrocnemius muscle, four general conditions were studied: 1, growth; 2, denervation atrophy; 3, fasting atrophy, and 4, atrophy following tenotomy.

Absolute muscle strength may be taken as the greatest tension developed in a short period of maximum effort. A number of factors, such as, quality and quantity of stimulus, resistance of the isometric lever in relation to maximum strength, initial tension on the muscle, species; size and functional state of the muscle, will affect the absolute magnitude of the tension. All these factors, except size and functional state, can be made constant. Results within a single series of experiments can be compared as to the functional state of the contractile elements if proper corrections are made for differences in size.

A muscle is composed of two general parts: 1, the contractile tissue proper, referred to as "muscle cell phase," and 2, the supporting and sustaining non-contractile elements referred to as "non-muscle phase." An approximation of the amounts of these two phases present in a muscle can be had from a chloride and water analysis of the tissue (Hastings and Eichelberger, 1937). For comparative purposes the tension values are best expressed per unit "muscle cell phase" since the relative amounts of these two phases are altered during growth and atrophy (Hines and Knowlton, 1937).

The rats used in these experiments were from closely inbred stock maintained on a standard ration of dog biscuits. Approximately equal numbers of males and females were used in each group. In experiments on the effect of growth, individuals from each litter were scattered among the various age groups studied. Adult rats between 6 and 12 months of age were used in the atrophy experiments. Denervation was accomplished by removing a section of the sciatic nerve to one gastrocnemius, the opposite gastrocnemius serving as the weight control. Rats for fasting were placed in individual cages and allowed water but no food. They were weighed daily until the desired weight loss was reached. Three levels of fasting

were used: *a*, short fast aimed at a muscle weight loss of 15 per cent; *b*, medium fast to a muscle weight loss of 25 per cent, and *c*, long fast leading to almost the maximum muscle weight loss possible without death of the animal. The initial muscle weights in these experiments were calculated from the initial body weights (Hines and Knowlton, 1934). Tenotomy was performed in adult rats by clipping off the os calcis, freeing the tendon from all bony attachments and sewing the cut end of the tendon into the skin. Thus, a length of tendon was preserved for making attachment to the isometric lever. The rat's tendon repairs rapidly so that at the end of two weeks it has formed new attachments which appear functional as early as three weeks after tenotomy. Six weeks after tenotomy the only gross difference in the appearance of the operated and control limbs is that the tendon of the tenotomized side is longer and the muscle shorter than on the control side.

Tension measurements were made with the animal under ether anesthesia. The gastrocnemius was exposed and measured, the length being taken from the point of origin at the internal condyle to the muscle-tendon junction. A portion of the femur was exposed and fixed in a rigid clamp, care being taken to avoid interference with the blood supply to the muscle. The tendon Achilles was cut and attached to a Blix type torsion rod. This rod and support is so designed that its resistance may be altered without changing the magnification. Thus regardless of the over-all muscle strength the extent of muscle shortening was kept between 0.05 and 0.1 mm. which, with optical recording, gave deflections of from 1.5 to 3 cm. on the film. The initial tension on the muscle was slightly less than that required to stretch it to its intact resting length which was measured with the foot midway between extreme extension and flexion. The muscle was stimulated through two silver electrodes which pierced the muscle, one at the tendon, the other at the origin. The maximal tension developed by the muscle in response to a short bout of condenser discharge shocks of supermaximal intensity and delivered at the rate of fifty per second was taken as the muscle strength. The peak voltage and frequency of these discharges were checked with a cathode-ray oscillograph before each experiment. After recording the tension developed by the muscle the animal was killed and the muscle removed and weighed.

Chloride and water concentrations were determined on muscles from similar experimental animals and these average values were used in calculating the muscle cell phase for each respective group.

The values obtained are expressed in relative terms in the accompanying table. In the experiments concerned with the effect of growth the absolute values for 180 day old rats were used as the base. Values for muscle undergoing atrophy and regeneration are expressed relative to the group labeled "adult animals" which were random samples of the same stock

used in the atrophy experiments. Muscle cell phases were calculated from water and chloride analysis, the muscle cell phase cross-section being the relative weight of the muscle cell phase divided by the relative length

TABLE 1

Effects of growth and atrophy upon muscle strength

CONDITION	RELATIVE MUSCLE				TENSION PER		NUMBER OF ANIMALS
	Weight	Muscle-cell phase	Length	Muscle-cell phase cross-section	Gram weight	Unit muscle-cell phase cross-section	
30 day normal	0.14	0.11	0.55	0.20	985	690	32
60 day normal	0.54	0.45	0.80	0.56	1,498	1,444	24
90 day normal	0.84	0.72	0.97	0.74	1,628	1,847	25
120 day normal	0.90	0.78	1.00	0.78	1,627	1,877	21
180 day normal	1.00	0.86	1.00	0.86	1,803	2,096	25
Adult normal	1.00	0.86	1.00	0.86	1,700	1,965	14
Adult atrophy							
3 days denervation	0.93	0.77	1.00	0.77	1,583	1,912	6
7 days denervation	0.80	0.64	1.00	0.64	1,502	1,878	12
14 days denervation	0.52	0.38	1.00	0.38	965	1,326	9
21 days denervation	0.38	0.26	1.00	0.26	606	886	12
28 days denervation	0.29	0.18	1.00	0.18	491	791	12
Short fast	0.85	0.72	1.00	0.72	1,703	2,010	8
Medium fast	0.75	0.61	1.00	0.61	1,621	1,993	9
Long fast	0.62	0.52	1.00	0.52	1,529	1,823	6
7 day tenotomy	0.83	0.67	0.73	0.92	1,435	1,294	6
14 day tenotomy	0.71	0.57	0.84	0.68	1,091	1,139	7
21 day tenotomy	0.78	0.65	0.84	0.77	1,236	1,252	6
42 day tenotomy	0.78	0.65	0.84	0.77	2,060	2,087	10

of the muscle. The tension per unit muscle cell phase cross-section is expressed on the basis of the relative values and calculated as follows:

$$\frac{\text{Tension in grams per gram wet weight} \times \text{relative weight}}{\text{Relative cross-section of muscle cell phase}} = \text{tension per unit relative cross-section of muscle cell phase}$$

This is regarded as the significant measure of the contractile strength per unit size.

The effect of growth was followed from an age of 30 to 180 days. It will be seen from the table that accompanying the increase in muscle length

there is an increase in the strength of the contractile mechanism. After 90 days of age, when the muscle length is 97 per cent of its final length, there was no significant increase in muscle strength per unit size.

Little change occurs in the strength of muscle during the first week following nerve section. Within this period of time the gastrocnemius muscle loses 20 per cent of its weight. After this there is a progressive and severe loss of strength indicating functional damage of the remaining muscle cell phase.

A muscle weight loss of over 40 per cent cannot be achieved by a food fast because of the death of the animal. However, these experiments indicate that in weight losses up to 38 per cent the muscle cell phase remaining retains its normal contractile strength.

Tenotomy gives a composite picture due to the eventual tendon and muscle regeneration. One week after tenotomy, at a muscle weight loss of only 20 per cent, there is severe functional impairment of the remaining muscle cell phase. This damage appears to be greater after another week, but then, as the new tendon becomes functional there is some regeneration of muscle substance and repair of damaged elements. Finally (6 weeks after tenotomy) there is a complete recovery of the contractile strength per unit cross-section of the muscle cell phase. This repaired muscle is, however, permanently lighter in weight and shorter in length than its control muscle.

It appears, from these results, that a major factor in the development and maintenance of the contractile strength of skeletal muscle is the constant stretch to which an intact muscle is subject by virtue of its insertion and attachment to the skeletal system. This passive stretch is enhanced by the continuous postural and occasional gross reflexes. That the increasing strength of skeletal muscles in growing animals is probably influenced considerably by the stretch which the growing skeletal system places on the muscle is shown by the fact that the period of greatest increase in strength per unit size coincides with the period of rapid increase in the length of the long bones. In tenotomy the passive stretch and effectiveness of reflexes is abolished for a time. Here we find a pronounced loss of muscle strength. After denervation the effect of passive stretch remains while active stretch due to reflexes is abolished. This also leads to loss of muscle strength, but for a given weight loss this is not as severe as in the tenotomy experiments. In fact, at a weight loss of twenty per cent the denervated muscle shows little if any loss of strength while the tenotomized muscle function is severely impaired. Contrasted with these two types of atrophy, fasting causes no apparent decrease in the muscle strength per unit of functional cross-section although the weight loss is at about the same rate as in denervation.

SUMMARY AND CONCLUSIONS

The method of expressing tension developed by a muscle in terms of unit cross-section of muscle-cell phase (chloride free material) is used in the belief that it gives the best comparative measure of the instant physiologic state of the contractile mechanism.

It was found that in the normal rat the full strength of the contractile mechanism per unit size was realized at about 90 days of age.

Weight loss in denervation atrophy was found to proceed to about twenty per cent without loss of strength per unit muscle-cell phase. Following this a progressive and severe loss in the strength of the remaining muscle-cell phase occurred.

Fasting atrophy was accompanied by no loss of strength in the remaining muscle-cell phase.

Atrophy following tenotomy was accompanied by a severe impairment of the contractile strength of the muscles. This change was found to be reversible following spontaneous tendon repair.

From this it appears that a major factor in developing and maintaining the contractile strength of the muscle is the physiologic stretch produced by the attachment of the muscle to the bony skeleton, this being enhanced by the postural and gross-movement reflexes.

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THE RELATION OF LABYRINTHECTOMY TO SENSITIZATION OF THE NICTITATING MEMBRANE IN THE CAT

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Camis (1) reported that the destruction of one labyrinth in the cat caused pronounced miosis and partial paralysis of the eyelids and nictitating membrane on the same side. De Kleyn (2) and de Kleyn and Socin (3) confirmed the occurrence of these symptoms and, on the basis of anatomico-physiological evidence, attributed them to the functional loss caused by the interruption of ocular sympathetic paths running through the middle ear. This view was not accepted by Camis (4). Since he found that the ocular syndrome followed destruction of the labyrinth even though the middle ear remained undamaged, he concluded (ibid. p. 202) that "the nerve-fibres crossing the promontorium of the tympanic cavity play no essential part in the pupillo-motor changes originating from the vestibule." Such a concept is in agreement with the work of Spiegel (5), which showed that stimulation of the labyrinth produced pupillary changes. It is evident that the part played by the labyrinth in the sympathetic control of the eye requires further investigation.

In this study we have attempted to apply the well-known observation that denervation of the nictitating membrane increases its response to intravenously injected adrenalin. If labyrinth destruction causes the ocular symptoms mentioned above because of the interruption of sympathetic pathways, the operation should be followed by sensitization of the nictitating membrane. On the other hand if some vestibular mechanism is concerned, the reaction of the membrane should be the same after section of the eighth cranial nerve as after labyrinthectomy. The response of the nictitating membrane to injected adrenalin has been tested before and after these operations.

METHODS. All operations were performed with strict aseptic precautions on cats anesthetized with nembutal (0.55 cc. per kgm. intraperitoneally). Although a few of the earlier labyrinthectomies were carried out by the method of de Kleyn (6), most of the animals were prepared by Camis' (7) occipital approach, which has the merit of avoiding injury to the cervical sympathetic nerve. Labyrinth destruction was verified by the

appearance of the well-known symptoms reported by Wilson and Pike (8) Magnus and de Kleyn (9), and others. The eighth nerve was sectioned intracranially through a trephine opening posterior to the bony tentorium and above the petrous portion of the temporal bone. Although the seventh cranial nerve is divided by the above procedure, the results obtained in this study indicate that this injury is not related to our problem. The picture presented by these animals was in agreement with that described by Bechterew (10) and Magnus and de Kleyn (9). Division of the eighth nerve was demonstrated at autopsy.

Contractions of the nictitating membranes were induced by the intravenous injection of 1.0 cc. of freshly prepared 1:50,000 adrenalin (Parke, Davis) while the animals were under nembutal anesthesia. The results were recorded on a kymograph by means of an isotonic muscle lever with a magnification of ten to fifteen times. Under these conditions the simultaneous responses of the two membranes of a normal cat sometimes differed as much as one hundred per cent. A greater variation than this was taken to be evidence of sensitization of the more reactive nictitating membrane.

Results. Unilateral destruction of the labyrinth was followed in all cases by protrusion of the nictitating membrane and constriction of the pupil on the side of the lesion. Experiments on nine cats in which the nictitating membranes were tested three to eighty-one days after operation showed that the contraction on the operated side was 2 to 11 times greater than on the normal side. Such a difference was never observed in unoperated animals.

A second series of experiments was carried out in which seven to ninety-eight days after unilateral labyrinthectomy the remaining labyrinth was destroyed and the response to injected adrenalin immediately recorded. In six cats prepared in this manner the nictitating membrane on the side of the older lesion gave contractions 3 to 9 times as large as those of the other side (fig. 1). Since the two sides differed only in the time elapsed following destruction of the labyrinth, the discrepancy observed indicated sensitization.

The degree of sensitization produced by labyrinthectomy was compared with that caused by excision of the opposite superior cervical ganglion. Twenty-one days after labyrinth destruction and seven days after superior cervical ganglionectomy, the responses of the nictitating membranes differed by less than twenty per cent (fig. 2). A second animal, seven days after labyrinthectomy and one year after extirpation of the ganglion, showed even less difference. These experiments indicate that the sensitization which follows destruction of the labyrinth is as great as that caused by interruption of the post-ganglionic sympathetic fibers.

The rate of development of sensitization following labyrinthectomy has

been studied in a number of cats. On the third postoperative day the response of the membrane was 3.6 times that of the control side; 2.2, on the fourth day; 2.5, on the fifth; 4.8, on the sixth; 3.8, on the eighth; and 2.7, on the thirty-fourth day. In any one cat the height of contraction did not decrease once a maximum for that animal had been attained. The above series of observations suggests that maximum sensitization has probably occurred as early as the third day after labyrinthectomy.

Further information was secured from experiments in which one labyrinth was destroyed at least ten days after the other. At various intervals after the second operation the responses of the nictitating membranes were recorded. Agreement between the heights of contraction of the two membranes should then show that maximal sensitization had been at-

Fig. 1

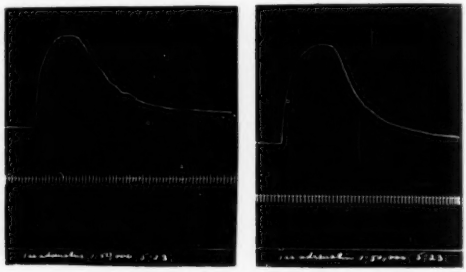

Fig. 2

Fig. 1. The simultaneous responses of the nictitating membranes to the intravenous injection of adrenalin (1.0 cc. of 1:50,000). Left eye—seven days after labyrinthectomy, right eye—two hours after labyrinthectomy. Time—10 seconds.

Fig. 2. The simultaneous responses of the nictitating membranes to the intravenous injection of adrenalin (1.0 cc. of 1:50,000). Right eye—7 days after section of the right cervical sympathetic nerve. Left eye—27 days after left labyrinthectomy. Time—10 seconds.

tained on the side of the more recent lesion. Estimated in this way, sensitization had not occurred on the day following the destruction of the second labyrinth. After the second day the contractions of the two membranes were approximately equal, indicating that sensitization was complete.

Hampel (11) states that objection can be offered to the use of a single standard dose of adrenalin on the ground that such a dose might be maximum at the level of greatest sensitivity of the muscle. In such a case the time required for the development of maximum sensitivity would be less than the actual value. In view of this statement the last series of observations was repeated on eight cats with graded doses of adrenalin. The dilutions used were 1:50,000, 1:100,000, 1:200,000, and 1:500,000.

The increased response of the membrane appeared to be maximal by the third postoperative day. This rather sudden development of sensitization, while in agreement with our earlier findings just discussed, is more rapid than that found by Hampel (11) after removal of the superior cervical ganglion. The reason for this discrepancy is not clear.

In two cats attempted destruction of one labyrinth was followed by miosis and protrusion of the nictitating membrane on the same side. None of the usual signs of labyrinthectomy (head rotation, head and eye nystagmus, falling to the side of the lesion, etc.) were observed. One of these animals was tested with adrenalin in the usual manner and the presence of sensitization demonstrated. It is suggested that in these two cases the

Fig. 3

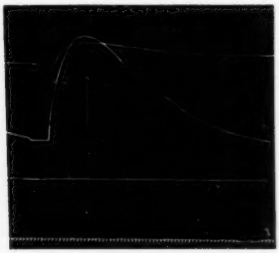

Fig. 4

Fig. 3. The simultaneous responses of the nictitating membranes to the intravenous injection of adrenalin (1.0 cc. of 1:50,000). Right eye—upper—6 days after intracranial section of the right VIII nerve. Left eye—lower—normal. Time—4 seconds.

Fig. 4. The simultaneous responses of the nictitating membranes to the intravenous injection of adrenalin (1.0 cc. of 1:50,000). Right eye—lower—6 days after right labyrinthectomy. Left eye—upper—24 days after intracranial section of the left VIII nerve. Time—4 seconds.

post-ganglionic fibers crossing the promontorium of the tympanic cavity were severed without injury to the labyrinth.

If the results of the above experiments were produced by the interruption of sympathetic fibers passing through the middle ear, the exclusion of labyrinth function by section of the eighth cranial nerve should not have the ocular effects mentioned above. This operation was performed on five cats. Five to eleven days after division of one eighth nerve the response of the homolateral nictitating membrane to injected adrenalin did not differ appreciably from that on the control side (fig. 3). The operation, therefore, does not produce sensitization.

These findings were confirmed by comparing the results of eighth nerve section with those of labyrinth destruction in the same animal. Nineteen days after section of the nerve and five days after destruction of the op-

posite labyrinth the contraction on the side of the labyrinthectomy was 5.6 times that on the contralateral side. A second animal (fig. 4) gave a response which, on the side of the labyrinth destruction (13 days), was 4.7 times that on the side of the eighth nerve section (24 days). It is important to note that the ocular symptoms which follow labyrinthectomy did not occur after the eighth nerve section. Miosis and protrusion of the nictitating membrane were never observed after intracranial section of the nerve. The contrast was especially striking in those animals which had suffered both lesions.

Discussion. The results of this investigation are in agreement with de Kleyn's (2, 3) anatomical and physiological studies concerning the course of the sympathetic fibers to the eye. According to this author, labyrinth destruction in the cat severs the post-ganglionic fibers which pass through the middle ear. It is this interruption of the sympathetic pathway to the eye that produces the sensitization of the nictitating membrane which we have described. On the other hand eighth nerve section, which leaves the sympathetic fibers undamaged, causes neither sensitization, nor ocular signs of sympathetic paralysis (miosis and protrusion of the nictitating membrane). It is evident that the ocular symptoms which are present after labyrinth destruction are to be attributed to the incidental interruption of sympathetic fibers running through the middle ear.

This relation of the sympathetic fibers to the middle ear has been reported by Meltzner and Wölfflin (12) in a study of the rabbit pupil. François-Franck's (13) anatomical investigations reveal a similar situation in the dog. Some sympathetic fibers connected with the iris probably run through the middle ear in man, for Dieter (14) has noted that after radical mastoidectomy, functional loss of the oculo-pupillary fibers of the sympathetic on the operated side is occasionally found. Curcio (15), who has observed similar ocular changes among the symptoms of acute and chronic otitis media, believes them to be of clinical significance.

SUMMARY

1. Unilateral destruction of the labyrinth in cats produced homolateral protrusion of the nictitating membrane and constriction of the pupil.

2. After labyrinthectomy the homolateral nictitating membrane was sensitized to injected adrenalin. The degree of the sensitization was comparable with that caused by removal of the superior cervical ganglion. Maximum sensitization was attained the third day after operation.

3. Miosis and protrusion of the nictitating membrane were not present after section of the eighth cranial nerve. Division of the eighth cranial nerve produced no sensitization of the nictitating membrane.

4. It is concluded that the ocular phenomena which follow labyrinthec-

tomy in the cat are the result of the incidental interruption of sympathetic fibers running through the middle ear.

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THE EFFECT OF HEAT ON THE GONADOTROPIC PITUITARY ANTAGONIST

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The property of anterior pituitary preparations to act as a gonadotropic antagonist upon intraperitoneal administration has raised the question of the existence of a separate "antagonist." In 1936 Evans and his collaborators reported the separation of an antagonist factor from the other gonadotropic hormones of the pituitary and thought it to be a distinct principle (1). In recent publications it has been shown, however, that there is, at present, no justification for the assumption of a separate antagonist pituitary factor apart from the luteinizing (LH) or interstitial cell stimulating (ICSH) principle (2, 3). Fevold and Fiske have also expressed the view that the antagonist factor is probably identical with the LH or ICSH (4). However, the question of the separate existence of a pituitary gonadotropic antagonist has been reopened by a recent publication of Bunde and Hellbaum (5). These authors reported that the luteinizing activity of various pituitary preparations is destroyed by heating in a boiling water bath while the antagonist activity remains apparently unaffected under these conditions, and concluded that the antagonist and luteinizing principles are not identical. We have carried out similar experiments with several pituitary preparations and observed partial destruction of the antagonist, luteinizing and interstitial cell stimulating properties. These results do not support the assumption that the pituitary secretes a separate antagonist principle.

EXPERIMENTAL. A 40 per cent alcohol extract from whole sheep pituitary, containing both FSH and LH (ICSH), and two purified LH fractions, used in these studies, were prepared and standardized according to the method published recently (2, 3). For the heat inactivation, aqueous solutions of these preparations were adjusted to pH 6 and placed in a boiling water bath for various periods of time. No appreciable flocculation was observed at the concentration employed (1 mgm. in 1.5 cc.). The solutions were adjusted to pH 7.4 and diluted for biological assay.

The unheated and heated solutions were tested for their antagonist properties in normal immature female rats by determining the counter-

action on the gonadotropic potency of either pregnant mare serum or 40 per cent alcohol extract from sheep pituitary. In the case of pregnant mare serum, the antagonist fraction was mixed *in vitro* with a standard dose of 1 mgm. of a purified pregnant mare serum preparation and given intraperitoneally. When a pituitary preparation was used as a gonad stimulator, the antagonist fraction was given intraperitoneally concurrently with the subcutaneous administration of 3 mgm. of the sheep pituitary material. Injections were made once daily for 3 days with autopsy 72 hours after onset of treatment. We have used a large number of animals (12 to 15) for each dose level when testing the antagonism against pregnant mare serum since the ovarian response to pregnant mare serum is very variable (3). In the antagonist tests against sheep pituitary extracts, 6 animals were generally employed for each dose level.

The luteinizing property of the unheated and heated preparations was tested by the degree of synergism obtained when given subcutaneously in combination with FSH. An *in vitro* mixture of both factors was injected once daily for 3 days and the resultant ovarian weight and development determined at autopsy 72 hours after onset of injection. Three normal immature rats were used in each group. In the same way, the FSH activity of unfractionated sheep pituitary extracts was determined by the synergistic effect with chorionic gonadotropin. In order to correlate the antagonist effect with the ICSH, the latter activity was determined in hypophysectomized immature rats by intraperitoneal injection.

Discussion. The results recorded in table 1 show that the antagonist property of the sheep extract, given at 1 mgm., is lost after heating for 30 minutes. The FSH activity of 40 per cent alcohol extracts is also destroyed by heating as can be seen from the augmentation tests with chorionic gonadotrophin (follutein). In hypophysectomized rats, the heated preparations were found to be devoid of gonadotropic activity at 1 and 0.5 mgm. in contrast to the hypertrophy of the interstitial tissue and follicular stimulation produced by the unheated material at these dose levels. It may be mentioned in this connection that a purified FSH preparation is apparently completely inactivated by heating for 30 minutes under the same experimental conditions while purified ICSH preparations are only partly destroyed under these conditions. The possibility of a differential rate of destruction of the two factors may have a bearing on the biological response of heated unfractionated sheep pituitary extracts.

The partial destruction of the antagonistic property of a purified ICSH, tested against pregnant mare serum, is illustrated in table 2. Similar results were obtained with another ICSH preparation (M.E.D. 0.025 mgm.). Parallel with the disappearance of the antagonism we observed a destruction of the ICSH effect in hypophysectomized rats. The partial loss of the antagonist property of a heated ICSH could also be demon-

strated against the gonadotropic activity of an unfractionated sheep pituitary extract (table 3). The antagonist effect of ICSH against pituitary gonadotropic preparations is less pronounced than that against pregnant mare serum. Whether the more sensitive antagonist response of ICSH

TABLE 1

*Heat inactivation of 40 per cent alcohol extract from sheep pituitary**

PREPARATIONS	OVARIAN WEIGHTS
	mgm.
I	
Pregnant mare serum	125
Sheep extract	18
Sheep extract + PMS	35
Sheep extract heated 30 min. + PMS	107
II	
Follutein, 10 RU	30
Sheep extract	23
Sheep extract + follutein	102
Sheep extract heated 30 min. + follutein	35
Sheep extract heated 20 min. + follutein	33
Sheep extract heated 10 min. + follutein	33

* One milligram total dose given either alone or combined in vitro with 1 mgm. of pregnant mare serum or 0.05 mgm. of follutein.

I. All injections given intraperitoneally. II. All injections given subcutaneously.

TABLE 2

Heat inactivation of ICSH[1]

Antagonism tested against pregnant mare serum[2]

ICSH UNHEATED	OVARIES	ICSH HEATED ½ HOUR	OVARIES	ICSH HEATED 2 HOURS	OVARIES
	mgm.		*mgm.*		*mgm.*
PMS alone	104				
PMS + ICSH 20 RU	27			PMS + ICSH 20 RU	57
PMS + ICSH 10 RU	38	PMS + ICSH 10 RU	55	PMS + ICSH 10 RU	80
PMS + ICSH 5 RU	48	PMS + ICSH 5 RU	75	PMS + ICSH 5 RU	100
PMS + ICSH 2 RU	72	PMS + ICSH 2 RU	100		
PMS + ICSH 1 RU	91				

[1] ICSH preparation, M.E.D. = 0.05 mgm. in hypophysectomized rats.

[2] PMS 1 mgm. total dose given intraperitoneally either alone or mixed in vitro with ICSH.

against pregnant mare serum is of any physiological significance is rather doubtful.

The luteinizing property of an unheated and heated ICSH solution was tested by *in vitro* combination with FSH, administered subcutaneously

(table 4). Although the unheated ICSH produces hardly any increase in ovarian weight in combination with FSH at the dose levels employed, synergism is indicated by the appearance of corpora lutea in the ovaries of all animals receiving both factors. Five RU of the heated ICSH combined with FSH also produced corpora lutea in the ovaries of all three animals. But at the 2.5 RU level, only one animal had corpora lutea

TABLE 3

Heat inactivation of ICSH[1]

Antagonism tested against sheep pituitary extract[2]

ICSH UNHEATED	OVARIES	ICSH HEATED ½ HOUR	OVARIES	ICSH HEATED 2 HOURS	OVARIES
	mgm.		*mgm.*		*mgm.*
Sheep extract alone	47				
Sheep + ICSH 20 RU	27	Sheep + ICSH 20 RU	26	Sheep + ICSH 20 RU	53
Sheep + ICSH 10 RU	29	Sheep + ICSH 10 RU	40	Sheep + ICSH 10 RU	48
Sheep + ICSH 5 RU	32	Sheep + ICSH 5 RU	48		

[1] ICSH preparation, M.E.D. = 0.05 mgm. in hypophysectomized rats.

[2] Forty per cent alcohol extract from dried sheep pituitary, 3 mgm. total dose given subcutaneously, ICSH given intraperitoneally.

TABLE 4

Heat inactivation of ICSH[1]

As tested for its luteinizing property in combination with 8 RU of hypophyseal FSH[2]

ICSH UNHEATED	OVARIES	DIAGNOSIS	ICSH HEATED ½ HOUR	OVARIES	DIAGNOSIS
	mgm.			*mgm.*	
FSH alone	32	m + ml F			
FSH + ICSH 5 RU	37	lF, CL	FSH + ICSH 5 RU	29	m + lF, CL
FSH + ICSH 2.5 RU	25	lF, CL	FSH + ICSH 2.5 RU	28	mF, CL
FSH + ICSH 1.25 RU	32	lF, CL	FSH + ICSH 1.25 RU	28	m + mlF

[1] ICSH preparation, M.E.D. = 0.05 mgm. intraperitoneally in hypophysectomized rats. M.E.D. = 0.2 mgm. subcutaneously in hypophysectomized rats.

[2] FSH preparation, M.E.D. = 0.05 mgm. subcutaneously in hypophysectomized rats, given subcutaneously either alone or combined in vitro with FSH.

Abbreviations: F, follicles; m, medium; ml, medium large; l, large; CL, corpora lutea.

and the 1.25 RU level failed to show any luteinization. These findings indicate that the luteinizing effect is also partly destroyed by heat.[1]

The observations recorded in this paper indicate that heating destroys the multiple physiological properties of the pituitary ICSH principle at

[1] It should be pointed out that the M.E.D. for the ICSH effect in hypophysectomized rats is 4 to 5 times higher on subcutaneous injection compared with intraperitoneal administration. In tables 2 and 3 (antagonism), the potency of the preparation is expressed in intraperitoneal ICSH units while in table 4 (luteinization) the potency is given in subcutaneous ICSH units.

approximately the same rate. By standardizing the antagonist and luteinizing effects of heated and unheated fractions at different dose levels, it is possible to demonstrate partial destruction for both effects. Moreover, the gradual loss of antagonist potency, by heating, parallels a corresponding increase in the M.E.D. for repair of the interstitial tissue in hypophysectomized animals. Our findings, therefore, do not support the claim of Bunde and Hellbaum (5) that heat will destroy only the luteinizing property without influencing the antagonist effect of hypophyseal LH (ICSH) fractions. The findings recorded in this paper are in agreement with the identity of the antagonist and ICSH (LH) of the anterior pituitary.[2]

SUMMARY

Various gonadotropic preparations from sheep pituitary have been subjected to heat treatment and their gonadotropic response studied in normal and hypophysectomized immature female rats. The FSH apparently is less stable against heating than the ICSH (LH) fraction. The multiple physiological properties of the ICSH fraction (repair of the interstitial tissue, luteinization and antagonism) are destroyed by heating at approximately the same rate.

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[2] It should be emphasized that the hypophyseal LH (ICSH) differs chemically and biologically from the luteinizing principles from pregnant mare serum, chorionic gonadotropin (pregnancy urine), gamone (menopause urine) and prospermine (normal male urine). The antagonist property is observed only with the pituitary LH (ICSH) (3).

THE WATER CONTENT OF VARIOUS ORGANS OF THE ALBINO RAT ON A HIGH CARBOHYDRATE AND A HIGH FAT DIET

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Clinical observations seem to indicate that a diet containing a large percentage of carbohydrate increases the hydration of the tissues, whereas a diet poor in carbohydrate but rich in fat has a dehydrating effect (1, 2). The retention of water on a high carbohydrate diet, it is believed, does not occur in the blood but in the skin and other tissues (2). Because of the indirect nature of the evidence upon which these conclusions are based, it was deemed advisable to reinvestigate the problem by a more direct approach. In the present investigation quantitative determinations were obtained on the water content of various organs of non-exercising and exercising albino rats fed a high carbohydrate and a high fat diet. Similar determinations were also made on a group of animals maintained on a stock diet to serve as a norm of comparison for the data obtained on the carbohydrate and fat diets. The stock diet was regarded as representative of the diversified food intake of man on a normal, balanced diet.

METHOD. Inbred albino rats of the Wistar stock were used throughout the experiments. The animals were divided into five groups with fourteen (7 males and 7 females) in each group, or a total of 70 animals. Three groups, which will be referred to as the rest or non-exercising series, were kept in small cages measuring 17.5 × 20 × 27.5 cm.; two groups constituting the exercise series were kept in revolving cages. Each end of the shaft of the exercise wheel of the revolving cage was attached to a Veedor rachet counter so that both clockwise and counter-clockwise rotations were recorded. The counters were read daily.

The animals were placed at weaning on the Wistar stock diet and ten days later on the experimental diet. In the rest series there were three groups that were fed, respectively, a high carbohydrate, a high fat, and a stock diet, and in the exercise series two groups that were fed the same carbohydrate and fat diet as the rest animals. Each animal on the carbohydrate diet was a litter mate of one on the fat diet.

The high carbohydrate diet contained 70 per cent sucrose and 15 per cent

casein; the high fat diet 50 per cent pure lard, 20 per cent sucrose and 15 per cent casein. In addition, each contained 5 per cent Osborne-Mendel salt mixture and 10 per cent Fleischman's brewers yeast. The stock diet was made by mixing 1 quart barley, 1 quart wheat, 4 cans tomatoes, 2 cans string beans and 2 cans salmon. Sodium chloride was added, the mixture thickened with corn meal and then cooked with frequent stirring. When cooled it was passed through a sausage grinder and dried. The animals fed on this diet were given also Purina dog chow daily, lettuce or cabbage once a week and milk and hard boiled eggs twice a week. All the animals were given daily by medicine dropper 7 drops of a 6:1 mixture of cod liver oil and linoleic acid.

The animals were allowed to eat and drink (distilled water) *ad libitum*. They were kept on the rations for 70 days and at the conclusion of this period fasted for 24 hours during which time they had free access to water. They were then decapitated and the body held suspended by the tail until bleeding stopped. Dissection followed immediately in a room saturated with water vapor. The heart was cut open and the blood, which was usually clotted, removed. Since the muscle tissue constitutes such a large portion of the body weight, it was deemed advisable to determine separately the water content of both the right and the left gastrocnemii. The separate determinations on the symmetrical muscles served as a check on the method. Fat and other extraneous tissue adhering to the organs were carefully dissected away. The different organs were placed in separate weighing bottles, weighed, dried in an evacuated desiccator for 48 hours over $CaCl_2$ and finally to constant weight over P_2O_5.

Acetone bodies were determined by the Van Slyke gravimetric method (3) on 78 twenty-four hour collections of urine obtained from different animals in the non-exercise and exercise groups on the three diets under both fasting and non-fasting conditions. During the period in which the urine was collected, the animals were kept in glass jars, one foot in diameter, having a funnel-shaped bottom of tin covered with a wire mesh. The urine was collected in a beaker under oil.

Results. The averages obtained from the data of these experiments together with the standard deviations of the variates are presented in table 1. The statistical significance of each difference between the water content of corresponding organs of the animals on the different diets was obtained by applying the t test of Fisher (4). Two procedures were followed in calculating the value of t. When the animals were litter mates, the formula $t = \frac{\bar{x}\sqrt{n-1}}{S_x}$ was applied[1] to allow for the correlation between

[1] $S_x = \sqrt{\frac{\Sigma d^2}{n}}$.

litter mates (compare Fisher, p. 112); when they were not litter mates, the formula $t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{\Sigma d_1^2 + \Sigma d_2^2}{N_1 + N_2 - 2}}} \sqrt{\frac{N_1 \times N_2}{N_1 + N_2}}$ was used (compare Fisher, p. 114).

In order to conserve space the results of the statistical analysis are not presented in tabular form but will be referred to briefly in the text. The probability of a difference between two averages being a chance occurrence will be designated by the conventional P. When the value of P was found

TABLE 1

Percentage water content of various organs of non-exercising and exercising albino rats on different diets

ORGAN	NON-EXERCISE SERIES						EXERCISE SERIES			
	High carbohydrate diet		High fat diet		Stock diet		High carbohydrate diet		High fat diet	
	Water content	Standard deviation	Water content	Standard deviation	Water content	Standard deviation	Water content	Standard deviation	Water content	Standard deviation
	per cent		*per cent*		*per cent*		*per cent*		*per cent*	
Brain	77.4	±0.4	77.4	±0.6	77.9	±0.9	77.8	±0.6	77.8	±0.3
Liver	69.3	±0.8	66.4	±2.6	69.4	±0.4	69.6	±0.9	67.4	±1.3
Heart	76.7	±0.4	76.5	±0.6	76.3	±0.5	76.2	±0.5	76.7	±0.7
Lungs	78.6	±1.7	78.9	±1.5	79.1	±1.0	79.3	±2.8	78.7	±1.9
Muscle (right gastrocnemius)	74.9	±0.4	74.5	±0.7	74.3	±0.5	74.8	±0.6	74.3	±0.9
Muscle (left gastrocnemius)	75.1	±0.4	74.5	±0.4	74.4	±0.4	74.9	±0.6	74.2	±0.7
Kidneys (7 males)	75.3	±0.6	74.3	±0.4	75.7	±0.7	75.3	±0.9	74.8	±0.6
Kidneys (7 females)	76.1	±0.2	75.6	±0.7	76.3	±0.6	75.9	±0.9	75.7	±0.4
Spleen	75.7	±0.5	75.9	±0.3	76.7	±0.4	75.6	±0.8	76.2	±0.6
Testes	87.1	±0.3	87.2	±0.5	86.9	±0.4	87.7	±0.8	87.4	±0.2
Ovaries	74.2	±1.8	72.6	±1.9	73.9	±0.6	73.1	±1.9	71.6	±1.7
Thymus	74.5	±2.9	72.3	±2.6	76.1	±1.5	75.5	±2.5	73.0	±2.4
Adrenals	60.8	±3.2	60.9	±1.6	65.2	±1.9	63.2	±2.8	61.8	±2.8
Thyroid	72.1	±3.0	67.8	±3.1	71.2	±2.5	71.1	±5.8	70.3	±3.5
Pituitary	75.1	±9.5	75.2	±7.3	75.7	±3.4	76.8	±4.6	76.1	±2.6

to be greater than 0.05, the difference in water content on the two diets in question was taken to be of no significance. In most instances there will be no reference in the text under the caption of the individual organs to insignificant differences. It will therefore be understood that when no specific reference is made to differences between the average water content of corresponding organs on different diets, the differences were not significant.

The averages in the table, except for the gonads and the kidneys, were obtained from the combined data on the males and females. The kidneys

of the two sexes were treated separately as these organs showed a sex difference in the water content on both the carbohydrate and fat diets in the non-exercise series and on the fat diet in the exercise series. In these instances the percentage water content was, respectively, 0.8, 1.3, and 0.9 per cent higher in the males than in the females. These higher percentages were definitely significant as P was equal to 0.0085, 0.0013 and 0.0094, respectively. In all the other organs there was no significant sex difference. It is of interest to note that Donaldson (5) found no sex difference in the percentage water content of the organs of the rat when the animals were fed on a stock diet.

The factor of ketosis did not enter into the results as the urinary excretion of ketone bodies on the various diets was within the normal range both when the animals had access to food and when they were fasted twenty-four hours. The average twenty-four hour excretion on the different rations in the fasting and non-fasting state was less than 50 mgm. per square meter of body surface computed by Lee's formula (6). These values were of the same order as those found by Deuel and others (7) in their control rats. Our observations are therefore in agreement with those of Levine and Smith (8) who found the excretion of total acetone bodies by the rat to be independent of the proportion of fat, carbohydrate or protein in the diet.

Rest Series. Heart, lungs, gonads and pituitary. The difference in the water content of these organs on the three diets was not significant. In the case of the ovaries the differences were relatively large but they were nevertheless found to be of no statistical significance.

Brain. The larger percentage of water in this organ on the stock diet than on the carbohydrate and fat diets, although relatively small, was significant as P was equal to 0.0037 and 0.0146, respectively.

Liver. The lower percentage of water in the liver on the fat diet as compared with the water content on the carbohydrate and stock diets was significant, as P was equal to 0.004 in the first instance and 0.0005 in the second.

Muscle. The higher percentage of water in the muscle on the carbohydrate diet, although of small magnitude, was definitely significant. In the statistical comparison of the percentage water in the right and left gastrocnemii on the carbohydrate diet with that in the same muscles on the other two diets, the value of P ranged from 0.024 to 0.0006.

Kidneys. The kidneys of both the males and females on the fat diet, as shown in the table, contained less water than on the carbohydrate diet (P = 0.008 and 0.035, respectively), and also less than on the stock diet (P = 0.002 and 0.009, respectively). The smaller percentage on the fat diet as compared with the other two diets was therefore definitely significant.

Spleen. The percentage water content of the spleen was definitely higher on the stock than on the carbohydrate and fat diets, the value of P in each instance being less than 0.0001.

Thymus. The difference of 1.6 per cent in the water content of the thymus on the carbohydrate and stock diets was not significant, as P was equal to 0.088. This high value of P, notwithstanding the relatively large difference between the averages, as contrasted with the low value of P for smaller differences in the case of other organs such as the muscle, is accounted for by the relatively large standard deviation in the case of the thymus. The lower water percentage in the thymus on the fat diet as compared with both the carbohydrate and stock diets was definitely significant, as P was equal to 0.0334 and 0.0016, respectively.

Adrenals. These organs contained definitely less water on the carbohydrate and fat diets than on the stock diet (P = 0.0036 and 0.0002, respectively).

Thyroid. The relatively large difference in the water content of the thyroid on the carbohydrate and stock diets, as in the case of the thymus, was not significant. On the fat diet the percentage water was significantly lower than on the carbohydrate and stock diets (P = 0.017 and 0.0096, respectively).

Exercise Series. A statistical comparison of the rest and exercise experiments showed that exercise had no effect on the water content of the organs. In many cases the difference in the percentage water in the exercise and rest series was extremely small; in others the difference was relatively large, but in all cases except for the adrenals the value of P did not approach 0.05. The water content of the adrenals on the carbohydrate diet was 2.4 per cent higher in the rest than in the exercise animals, but since P was equal to 0.053 it is doubtful whether this difference was significant. It should be noted that the exercise animals were not litter mates of the rest animals on the same diet. Donaldson (5) in a similar study with litter mates on a stock diet found that after a rest of one day following 90 days in a drum cage, the percentage water in the organs was only slightly higher than in the organs of the non-exercising control animals. The data of his experiments, however, were not submitted to a statistical analysis. The males and females in our experiments ran, respectively, an average of 0.87 and 2.19 miles daily on the carbohydrate, and 1.53 and 3.03 miles on the fat diet. The males in Donaldson's experiments ran an average of 4.7 miles and the females 6.6 miles per day.

In the exercise animals of our experiments the same relationships were found between the water content on the carbohydrate and fat diets as in the non-exercising groups except for the spleen and the kidneys. In the rest series there was no significant difference in the percentage water of the spleen on the carbohydrate and fat diets; in the exercise series, however,

this organ contained 0.6 per cent more water on the fat than on the carbohydrate diet, the percentage being higher on the fat diet in 12 out of 14 experiments. The difference was definitely significant as P was equal to 0.007. The kidneys in the non-exercise animals contained definitely more water on the carbohydrate than on the fat diet. In the exercise experiments the kidneys of the males and females contained 0.5 per cent and 0.2 per cent more water, respectively, on the carbohydrate than on the fat diet, but it could not be shown statistically that these differences were significant (P = 0.09, and 0.12).

Discussion. The correct evaluation of the effects of a high carbohydrate and a high fat diet on the hydration of the various organs can be had only by comparing their water content on these diets with that which obtains with a balanced, diversified food intake, as on the stock diet in these experiments. If a comparison is drawn between the water content of the heart, lungs, gonads, pituitary, brain, spleen, and adrenals on the carbohydrate and on the fat diets only, it would appear as if neither diet had any effect on these organs. If, however, a further comparison is made with the data obtained on the stock diet, it will be observed that in the first four of these organs there was no significant difference on the three diets, while in the last three organs the percentage water was significantly lower on the carbohydrate and fat than on the stock diet. These comparisons lead to the conclusion that neither the carbohydrate nor fat diets had any effect on the water content of the heart, lungs, gonads, and pituitary, but both carbohydrate and fat had a slightly dehydrating effect on the brain, spleen, and adrenals. Similar comparisons of the data on the three diets in the case of the muscle, liver, kidneys, thymus, and thyroid show that, while in these organs the percentage water was higher on the carbohydrate than on the fat diet, in the muscle it was not significantly different on the fat and stock diets, and in the other organs it was not significantly different on the carbohydrate and stock diets. It may therefore be concluded that carbohydrate had a slight hydrating effect on the muscle and fat a dehydrating effect on the liver, kidneys, thymus and thyroid.

In view of the statement found in clinical literature that a high carbohydrate-low fat diet increases the water content of the tissues (2, 9, 10), it is of interest to determine the increase in body weight and size of the muscles that would be induced by an increase in the hydration of muscle tissue on the carbohydrate diet equal to that which was observed in the present experiments. A few simple calculations show that a rat weighing 250 grams, with the weight of the musculature equal to approximately 45 per cent of the body weight (11, p. 133) would gain only about 0.5 gram if the water content of the muscles were increased by 0.6 per cent, while the increase in the volume of the individual muscles would amount to approximately 0.5 per cent. If the same increase in hydration of muscle were induced in the muscles of man as in the rat by a high carbohydrate diet,

approximately 100 grams would be added to the body weight of an individual weighing 70 kilos. An increase of this magnitude would appear to be of no practical significance.

We wish to acknowledge our indebtedness to Mr. Winfrey Wynn and Mr. Charles Ensor for feeding and caring for the animals used in these experiments.

SUMMARY AND CONCLUSIONS

The effect of prolonged feeding of a high carbohydrate and a high fat diet on the water content of the organs of the albino rat has been determined on both non-exercising and exercising animals.

No sex difference was found in the percentage water of the various organs except in the case of the kidneys on both diets in the non-exercise series and on the fat diet in the exercise series.

Exercise had no effect on the hydration of the organs.

Taking the water content of the organs of the animals on a diversified (stock) diet as a norm of comparison, the following conclusions may be drawn:

1. Neither the carbohydrate nor the fat diet had any effect on the hydration of the heart, lungs, gonads, and pituitary.
2. Both diets had a dehydrating effect on the brain, spleen, and adrenals.
3. The carbohydrate diet produced a slight increase in the water content of the muscle whereas the fat diet had a dehydrating effect on the liver, kidneys, thymus, and thyroid.

Calculations from the data of these experiments show that the increased hydration of muscle tissue on the carbohydrate diet would apparently result in only a small increase in body weight and in the size of individual muscles. From a practical point of view the increase would appear to be of no consequence.

Ketosis played no part in the results of these experiments as the urinary excretion of ketone bodies on the fat, as on the other diets, was within the normal range.

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THE UTILIZATION OF PARENTERALLY ADMINISTERED HORSE SERUM BY THE RAT[1]

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Whipple and those who have collaborated with him (1, 2, 3) have shown that the dog can use for protein anabolism the proteins of dog plasma injected intravenously. The observations reported in this paper show that the rat can transform the proteins of horse serum into the proteins of its own organs and tissues when this foreign serum is injected intraperitoneally.

After one day's fast two groups of 4 ♂ rats were selected so that the average body weight of each group was 182 grams. In one group horse serum[2] was injected intraperitoneally in doses of 5 cc. per 100 sq. cm. of body surface daily and to the other group the same quantity of 0.9 per cent sodium chloride was given intraperitoneally. As food both groups took a 30 per cent dextrose solution that contained 0.1 per cent of sodium chloride and 1 per cent of a concentrate of rice polishings that contains all the B vitamines (Galen B concentrate). After 22 days both groups were killed and the total protein and the protein content of certain organs and tissues was determined (4). The results are given in table 1.

There were 29.67 grams of protein in the serum-injected group as compared with 24.51 grams in the sodium chloride group, a difference of 5.2 grams. Since it may be assumed that both groups had at the beginning the same total protein content this represents a sparing of 21 per cent of the body protein by the administration of the horse serum.

In the organs and tissues it will be noted that by far the greatest relative difference was in the serum of the drawn blood. This was all the blood that could be collected from the severed abdominal aorta while the heart was still beating and is ordinarily about 75 per cent of the total blood volume. The serum (obtained by centrifuging the coagulated blood at high speed for half an hour) was greater in weight by 179 per cent in the horse serum injected group and had 8.8 per cent protein as compared with 4.9 per cent in the controls. It thus came about that the protein of the

[1] This work was aided by a grant from the Rockefeller Foundation.

[2] We are much indebted to the Cutter Laboratory of Berkeley, California for a large quantity of sterile normal horse serum.

drawn serum was more than double that found in the sodium chloride group, an increase so much greater than that found in any other part of the body that it seems reasonable to ascribe it chiefly to the passive circulation of not yet utilized protein derived from the horse serum. But such an explanation cannot be advanced for the differences found elsewhere, for the remainder of protein rich serum left in the capillaries of the organs and tissues of the horse serum injected group did not contain more than 0.04 gram and the difference to be accounted for exceeds 5.0 grams. The conclusion that that quantity of protein had been built into and become a part of the tissue and organ proteins of the body seems to us to be inescapable.

In the last two columns of table 1 the distribution of the total protein between the various organs and tissues of the body is given. This

TABLE 1

	PROTEIN PER RAT		FRESH WEIGHT PER RAT		PROTEIN SPARED BY SERUM, GRAMS PER 100 GRAMS NaCl PROTEIN	PROTEIN DISTRIBUTION, GRAMS PER 100 GRAMS TOTAL PROTEIN	
	Horse serum	NaCl	Horse serum	NaCl		Horse serum	NaCl
	grams	*grams*	*grams*	*grams*	*per cent*	*per cent*	*per cent*
Total	29.669	24.507	173.000	133.500	21.0	100	100
Liver	0.783	0.601	7.958	3.940	30.6	2.64	2.45
Kidney	0.199	0.145	1.190	0.897	37.9	0.67	0.59
Heart	0.120	0.096	0.714	0.564	25.0	0.41	0.39
Serum	0.361	0.112	4.100	2.292	(222.3)	1.22	0.46
Clot	0.668	0.546	2.648	2.022	22.3	2.25	2.23
Alimentary tract, etc.	2.133	1.439	25.090	17.785	48.2	7.19	5.87
Carcass	25.405	21.568	131.300	106.000	17.9	85.63	88.01

measure (organ protein per 100 grams total protein) is independent of the final body weights of the two groups and of any differences in the fat and water content of their bodies. The most general difference between the horse serum and the sodium chloride group is that in the former a greater proportion of the total protein is assigned to the internal organs and less to the carcass (chiefly muscle, skin and skeleton). This is the principal difference in protein distribution found when rats given protein as food are compared with those deprived of all protein (5).

We have already shown that rat serum is used by the rat (6) just as dog plasma is used by the dog. But this experiment with horse serum shows that protein foreign to the rat is built into the protein structure of the animal without digestion in the intestine. The evidence as to the high degree of individuality with respect to the ordering of the amino acids

within each protein (7) would seem to preclude the possibility of the direct incorporation into the organ proteins of the rat of the products of any partial digestion of the horse serum proteins. The hypothesis has been advanced (6) that digestion to amino acids takes place within reticulo-endothelial cells, particularly those of the mesentery and the liver. We looked for a hyperplasia of these cells in the serum injected rats but if this occurs it is masked by the increase in lymphocytes, leucocytes and plasma cells consequent, presumably, to an irritation of the peritoneum and subperitoneal tissues by the horse serum. Other means must be found to test this possibility.

CONCLUSION

Horse serum proteins injected into the peritoneal cavity of the rat are utilized in the maintenance of the proteins of the organs and tissues of the body.

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THE VALIDITY OF THE ELECTRICAL DOUBLET THEORY OF THE CARDIAC ACTION CURRENT

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The observations and experiments of Lewis (1), of Craib (2, 3, 4), of Wilson (5) and his collaborators, and of Eyster et al. (6), and more recently of Macleod (7) have indicated that when muscle, immersed in solution, is invaded from end to end by a wave of excitation, the wave front is preceded by an anode, or source, and is followed by a cathode, or sink. According to Craib the source and sink are close together, thus constituting an electrical doublet (Craib) or electrical couplets (Wilson et al.). Various experimental procedures have been employed to prove the correctness of this view, but, with the exception of some of Craib's work, the evidence has not been wholly convincing. Most of the evidence may be interpreted either in accordance with the views of the authors named, or in accordance with a reformulation of the classical negativity theory.

When a muscle strip is immersed in an extensive solution and one galvanometer lead wire is connected to the center of the strip and the other to a distant point in the solution, an initial diphasic curve is recorded as the impulse invades the strip from end to end. According to the electrical doublet interpretation, the record shows that as the impulse approaches the contact on the muscle, there is an accelerating rise in potential at that contact. When the impulse reaches the contact, there is an abrupt reversal of polarity, so that the contact becomes strongly negative. This negativity, at first rapidly, then more slowly, declines, and finally disappears, and the contact is again nearly at the resting potential (fig. 1, *AC* and *BC*), although the muscle is still active. The remote contact is regarded as experiencing no appreciable change in potential.

It has been shown by Gilson and Bishop (8) that the presence of a moving electrical doublet or "sheet of doublets" is not the only possible explanation of this electrogram. They regard the resting muscle as positive (see their fig. 4). At rest, the distant contact, according to their view, would be at the same potential as the contact on the muscle. When an impulse is set up and travels toward the muscle contact, the active part of the muscle is electrically negative. As the surface area of negative (active) muscle grows, the surface area of positive (resting) muscle shrinks.

Consequently the potential of the solution, and, therefore, of the remote contact, falls. However, the contact on the muscle, if well shielded, remains at the potential of the resting muscle until the wave front of excitation reaches it. When this happens, the muscle contact promptly sinks to the potential of active muscle. The potential of the muscle contact is now lower than that of the remote electrode because the latter is at the average potential of the whole muscle surface. Soon, however, the whole strip has been invaded. Now the potential at the two contacts is equal and the curve has fallen back to zero. The initial diphasic deflection has been recorded.

If one refers to the papers of Wilson and his collaborators, and to those of Gilson and Bishop, it becomes apparent that the fundamental disagreement hinges on one question. Is every charge of one sign balanced

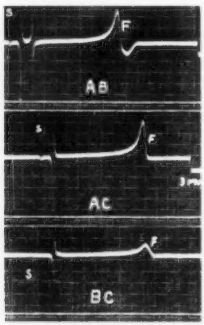

Fig. 1. Described in text. Time in 0.2 and 0.04 sec. The white dots are placed just below the peaks of the downward deflections. *S*, signal of stimulation. *F*, final deflections.

by an *equal* charge of the opposite sign, or may the charges be unequal? The former authors postulate balanced charges, and regard sources and sinks as being close together. Thus, from a given remote point in the solution, the distances to the positive and to the negative charges of each couplet, however oriented, are so nearly equal that the remote point is practically at the potential of the earth. Wilson, Macleod and Barker should be consulted for a mathematical analysis.

Gilson and Bishop assume that sources and sinks of unequal magnitude may exist. As the wave of excitation invades the muscle, the surface of the active muscle is negative, that of the inactive muscle, positive. The source shrinks as the sink grows. If this were true there is no doubt that the remote contact would experience potential changes. Although their view is competent to explain the records they choose for illustration, it is rendered untenable by the experimental results described below.

METHOD. A strip of muscle from the turtle ventricle was immersed in Ringer's solution in a large evaporating dish. The diameter of the solution surface averaged about 30 cm. The strip was sometimes driven by the attached sinus and auricle; sometimes by break induction shocks applied at the "proximal" end. The current was led from 3 points. One point was the uncut surface of the strip near its proximal end; one, the surface near the distal end; the third, a remote point in the solution, opposite the center of the strip and about 14 cm. distant. The electrodes were absorbent cotton wicks, connected to ordinary porous "boot" electrodes containing saturated $ZnSO_4$ solution and freshly amalgamated Zn strips. The ends of the wicks on the muscle were shielded, except at the muscle surface, by threading them into small glass tubes.

The records were taken on a Cambridge Instrument Company Mobile-All-Electric Electrocardiograph. No compensating current was used. Standardizations were in 3 mv. To eliminate demarcation currents, the muscle had been immersed for at least half an hour before the experimental records were taken.

In each record we will call the proximal contact A; the distal, B; and remote contact, C. *AB* was lead I of the electrocardiogram, relative negativity of A giving an upward deflection. *AC* was lead II, again negativity of A giving an upward deflection. *BC* was lead III, relative negativity of B yielding an upward deflection.

EXPERIMENTS AND INTERPRETATIONS. 1. Figure 1 shows the three leads from a strip electrically stimulated. Escape of current on break signals the time of stimulation. We shall first consider the potential changes occurring at contacts A and B during invasion of the strip by the wave of excitation.

In *AB* of the figure, the potential changes at B appear as the second diphasic effect; in *BC* the same diphasic curve stands alone, but the direction of the deflections is reversed. In *AC*, the deflections are the same as the first diphasic effect in *AB*. The time from stimulation to the beginning of the second diphasic effect in *AB* is 0.29 sec.; in *BC*, the corresponding time is 0.30 sec. The practical equality of the times, together with the presence of the first diphasic response in *AB*, demonstrates that between the signal of stimulation and visible response in *BC*, a wave of excitation was approaching contact B.

According to the negativity theory, the initial deflection from A in *AC* must be due to a fall in potential at C. Therefore, if this theory is valid, activation of a small area between the extreme proximal end of the strip and contact A is sufficient to produce a fall in potential at C, beginning 0.12 sec. after stimulation. Obviously then, in derivation *BC*, the fall in potential at C should begin when the same length of strip has been activated. This means that in *BC*, the descent of the first deflection

should appear at about 0.12 sec. after the signal of stimulation. Instead, the deflection begins at 0.30 sec. after the signal. Its failure to appear at the expected time is incompatible with the negativity theory.

Furthermore, since the area of strip activated before B is reached will be much greater than the area activated when A is reached, the first deflection recorded from B should greatly exceed in amplitude the first deflection from A. Instead of being greater, the amplitude is actually less in this experiment, being 4.8 mv., while that from A is 12.5 mv. For similar reasons, the second, upward, deflection from A should be much higher than the corresponding deflection from B. As a matter of fact, in the series of experiments from 6 hearts, the deflections were of approximately equal average amplitude. These facts are quite inexplicable on the basis of the negativity theory.

2. The record to be examined is *AB*. This shows the diphasic signal of stimulation promptly followed by a downward, then an upward, deflection. The line then descends nearly, but not quite, to the base line. There then appears an upward, followed by a downward, deflection.

This record is similar to figure d, plate I, obtained by Craib (3) from sartorius muscle. In his experiments, however, one end of the muscle projected from the solution to facilitate its stimulation. Such an arrangement will certainly somewhat distort the electrical field and the results might possibly be questioned on that ground, although we do not doubt their validity. Furthermore, in Craib's records, the final deflections were mixed with the initial, thus complicating the picture. It may be noted that in the turtle ventricular strip, the final deflections follow long after the initial (*F* in fig. 1).

In our experiment before stimulation the contacts A and B are on resting muscle. Then the proximal end of the strip is stimulated. According to the negativity theory, there should be no deflection until the wave of excitation reaches A, but for the sake of our present argument we may admit that the initial downward deflection is due to some effect such as that described by Bishop and Gilson (9). It cannot be due to a fall in potential at B, for this contact, according to the theory, will be at the potential of resting muscle. The first upward deflection then records the arrival of the "wave of negativity" at contact A. On the basis of the theory, *A should remain negative, and B should remain positive*, as the wave front travels from A to B. Therefore, the first and second upward peaks should be connected by a plateau. No such plateau appears in any experiment when electrodes A and B are well separated. The experiment is easily repeated, and the results thoroughly consistent. When sartorius muscle is used as in Craib's experiments a plateau is not to be expected on the basis of either theory, since in skeletal muscle the final deflections begin

before the initial deflections are complete and outlast the latter by only a short period.

In terms of the electrical doublet theory, the fall between the two upward peaks is necessary. At the low point between peaks the doubtlets are about half-way between A and B, but so far from each that the effects are slight.

Bishop and Gilson (9) criticise Craib's demonstration of an electrical dipole. They point out that the axis joining the plus and minus pole must be transversely oriented, i.e., across the muscle fiber membrane, whereas Craib postulates a longitudinally oriented dipole. We do not regard the objection as valid. Craib did not consider the precise orientation, but found the dipole behaved *as if* it was longitudinally arranged. Wilson, Macleod and Barker's theoretical discussion should resolve the difficulty.

SUMMARY

Two experiments on heart muscle strips are described, the results of which are incompatible with the negativity theory of the origin of action potentials, and which support the theory that the initial deflections are due to the presence of electrical doublets.

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THE RELATIONSHIP OF THE ANTERIOR PITUITARY AND THE ADRENAL CORTEX IN THE METABOLISM OF CARBOHYDRATE[1]

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That under certain conditions both the anterior pituitary and the adrenal cortex may influence carbohydrate metabolism has now been demonstrated. The trophic effect of the anterior pituitary upon the adrenal cortex is also well known and has led to the suggestion that most or all of the effects of the A.P.E. upon carbohydrate metabolism may be mediated via the adrenal cortex. But recent work, discussed below, indicates that certain of these effects of A.P.E. may be independent of the adrenal cortex. It has therefore become important to determine carefully the types of activity in carbohydrate metabolism which may be ascribed to each of these hormones and to investigate further any interrelation between them. To this end, the following experiments have been undertaken:

1. A comparison of the effects of A.P.E. with those of adrenal cortical hormone (C.E.) on the metabolism of normal rats fed glucose.

2. A determination of the effects of A.P.E., of C.E., and of a combination of the two extracts on the disposition of fed carbohydrate in adrenalectomized rats. These experiments have also required the determination of the effects of adrenalectomy alone on fasting carbohydrate levels, on the effects of A.P.E. on these levels, and on the disposition of fed glucose in the absence of other treatment.

METHODS. All rats used were young males of the white or hooded (Long-Evans) strains, weighing from 140 to 180 grams at the time of the experiments. No differences were detected in the results obtained on animals of the two strains.

Bilateral adrenalectomies were performed by the lumbar approach at

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[2] National Research Council Fellow in Medicine, 1938-1939.

one operation in each case. Determinations of fasting carbohydrate levels were made from 3 to 20 days and experiments in which glucose was fed from 3 to 10 days after the operations; no differences were ascribable to the length of time after operation within these limits. After adrenalectomy, the rats were maintained in good condition by the administration as drinking water of a solution containing 0.7 per cent sodium chloride and 0.3 per cent sodium bicarbonate. A dry diet high in carbohydrate was furnished ad lib. except during the fasting periods. In some of the experiments extra salt was given during the fasting periods (2 2 ml. injections of 1.4 per cent sodium chloride with 0.6 per cent sodium bicarbonate), since in the absence of food, the rats did not drink the usual amounts of the saline mixture furnished. This treatment has been found to preserve the normal levels of blood mineral constituents even during prolonged fasting (H. C. Harrison, unpublished). No differences in the carbohydrate levels resulted from this treatment, although the rats so treated withstood fasting in much better condition than those without it. No rats were used which had not at least maintained their pre-operative weights during the intervals preceding the experiments.

The fasted rats were fed glucose in amounts just greater than those expected to be absorbed in the 4 hour experimental periods, in each case in a volume of 5 ml. The concentration of glucose thus fed was from 15 to 25 per cent in most cases. The adrenalectomized rats did not always tolerate these large amounts of hypertonic solution, and any experiments in which diarrhea developed or the rats seemed otherwise in poor condition were discarded.

The usual procedure in these experiments was as follows: rats fasted 18 to 24 hours were fed glucose solutions by stomach tube without anesthesia or excitement. They were then placed immediately in a respiratory apparatus, and after a short equilibrium period determination of the oxygen consumption and of carbon dioxide production was begun. The respiratory data was obtained in each case over a period of approximately $3\frac{1}{2}$ hours. The apparatus described previously (15) was used in earlier work; in all later experiments, including all those on adrenalectomized rats, an open circuit Haldane apparatus was used. At the end of the respiration periods, the rats were anesthetized with amytal or nembutal (10 mgm. per 100 grams), and samples of gastrocnemius muscle and of blood (from the vena cava), the whole livers, and the gastro-intestinal tracts were removed for glycogen or glucose analyses. All analytical methods were those used previously (15, 16).

The extracts used in these experiments were the following: A.P.E. (saline) was a 2 per cent sodium chloride extract of beef anterior lobes clarified by Sharples centrifugation. One milliliter contained about 18 mgm. of organic solids; the glycostatic unit (16) was less than 1 mgm.

A.P.E. (alkaline extract) was a barium hydroxide extract prepared as described previously (17). It contained 12 mgm. per ml. of organic solids and assayed 1 unit of glycostatic activity in 0.8 mgm. A.P.E. (adrenotropic) was prepared by the Moon procedure (14) also from beef pituitaries. It contained 10 mgm. per ml. of organic solids; one Moon unit was 24 mgm; and a glycostatic unit was 2.5 mgm.

C.E. was Upjohn's adrenal cortical extract from which the alcohol had been removed by vacuum distillation. In a normal series of rats, crystalline corticosterone (Compound B, from Kendall) was also used.

One milliliter of the pituitary extracts was given i.p. to each rat one-half to one hour before the feeding of glucose. The dosages were therefore approximately 8 to 12 mgm. per 100 grams. When fasted rats were being treated, 1 ml. of A.P.E. (saline) was given divided in two equal parts, one at the start of the fast and the other 8 to 12 hours later. The adrenal extract was given in a total dose of 0.5 ml. in some series and of 3 to 5 ml. in others, the crystalline material in a dose of 1 mgm. per 100 grams; in some cases the preparations were injected i.p. one-half to one hour before the glucose was fed, in others injected or fed with the glucose.

For the calculation of the disposition of the absorbed carbohydrate, a comparable series of data on fasted rats was obtained and used as baselines. Oxidation of carbohydrate was calculated by the usual methods. For the calculation of non-protein R.Q.'s, an average value for nitrogen excretion of 3 mgm. per 100 grams per hour was assumed. In earlier work (in normal rats) determination of the nitrogen excretion during the experimental periods was made, but the values obtained for such short intervals were so variable that the assumption of a constant average value seemed more reliable. In any case, errors due to this assumption were small in the range of R.Q.'s encountered in these experiments.

As has been done previously by other investigators, the assumption was made for these calculations that muscle glycogen and blood and tissue glucose are distributed in 50 per cent of the body weight. While individual errors due to this assumption must be large, they should be similar for the averages in each group and so render comparisons valid between groups.

Results and discussion. The data obtained in these experiments on fasted animals are presented in table 1, those from fed animals in table 2. The calculated distribution of the fed carbohydrate in each series is presented in table 3, together with the percentage distribution (percent of absorbed carbohydrate oxidized and recovered) in each case.

In confirmation of previous work in normal rats, whole anterior pituitary extract, either saline or alkaline, appears to depress the oxidation of fed carbohydrate and to promote the deposition of muscle glycogen. These extracts are very active as glycostatic agents—that is, in maintaining the muscle glycogen levels and in depressing the apparent rate of

oxidation of carbohydrate in fasted hypophysectomized rats—, but are not very active adrenotropic agents. Since the action of the A.P.E. in fed rats would appear to parallel that of the glycostatic factor in fasted rats, and since the latter operates in the absence of the adrenals, it seemed improbable that adrenotropic preparations would have the same activity as the whole A.P.E. Surprisingly, the adrenotropic extract used was also very active in depressing oxidation and in promoting muscle glycogen deposition in fed normal rats. However, experiments with fasted hypophysectomized rats showed that this particular adrenotropic extract contained considerable amounts of the glycostatic factor, although it was less active in this respect than the whole A.P.E. Moreover, probably much more than the minimum effective dose had been given of both the whole

TABLE 1

Carbohydrate levels of adrenalectomized rats

	NUMBER OF ANIMALS	DAYS POST-OPER.	BLOOD SUGAR	MUSCLE GLYC.	LIVER GLYC.	OXYGEN CONSUMP.	R.Q.
			mgm. per cent	*mgm. per cent*	*per cent*	*ml. per 100 gm. per hour*	
I. Fasted 24 hours							
A. Adrenalect. rats							
1. Untreated	6	3-4	56	477	0.02		
2. Untreated, showing insufficiency	4	10-20	57	325	0.02		
3. Salt treated*	13	3-19	54 ±3.5	432 ±14	0.02	138 ±5.4	0.727 ±.003
4. Salt treated, with A.P.E.†	9	3-4	31 ±1.4	497 ±15	0.02		
B. Normal rats	15		72 ±3.2	524 ±10	0.03	154 ±1.9	0.725 ±.005
II. Unfasted							
A. Adrenalect. rats salt treated	7	4-28	97 ±3.7	533 ±29	2.31 ±0.55		
B. Normal rats	13		125 ±2 0	530 ±13	1.65 ±0.14		

* Salt given in drinking water, containing 0.7 per cent NaCl and 0.3 per cent $NaHCO_3$.
† Saline extract, total dose of 1 ml. given in divided doses during fast.

A.P.E. and of the adrenotropic; so that whether the adrenotropic hormone was the active agent could not be concluded from this comparison. The next step, therefore, was to compare the effects of cortical hormone itself with those of A.P.E. when administered under the same conditions.

Pure corticosterone given to fed normal rats produced a consistent reduction in the R.Q. and in the calculated rate of oxidation of the fed glucose, with variable increases in the deposition of carbohydrate. Adrenal cortical extract (Upjohn's), administered under identical conditions by Katzin and Long (12), was more consistently effective. The extract appeared to decrease oxidation considerably, and it increased liver glycogen deposition by 50 per cent and muscle glycogen to a smaller extent. The effects of the C.E. and of the A.P.E. in depressing carbohydrate oxidation

appear therefore to be similar, but the C.E. seems to be distinguished by its greater effect on liver glycogen deposition. Since the action of the adrenotropic extract can be attributed to either of these factors, a final decision upon the respective rôles of A.P.E. and C.E. cannot be made from this limited number of experiments on normal rats.

The experiments on adrenalectomized rats appear to establish the distinction between the actions of A.P.E. and of C.E. In these animals, saline A.P.E., which was markedly effective in normal rats, did not at all affect the oxidation of carbohydrate. Its only action seemed to be a diminution of liver glycogen with some increase in the proportion deposited in the muscles and left in circulation in the blood and tissue fluids. Large doses (3 to 5 ml.) of the adrenal cortical extract, as in normal rats, depressed carbohydrate oxidation to some extent; but it caused deposition of the unoxidized portion wholly as liver glycogen, without affecting the muscle glycogen values. A smaller dose of the extract (0.5 ml.) had no demonstrable effect in any case. When, however, a combination of the two hormones was administered, the usual effect of the A.P.E. was evident, with massive deposition of muscle glycogen. This effect was obtained with both the larger and smaller doses of C.E.—that is, even when the dose of C.E. used was quite without effect when given alone. For the depression of oxidation of fed glucose, then, A.P.E. apparently required the presence of some adrenal cortical hormone, but, it must be noted, not the active mediation of the cortex itself, in the sense of a stimulation of cortical secretion by the A.P.E. Moreover, the effect of the A.P.E. upon the muscle glycogen appears unique, while C.E. seems to affect only liver glycogen deposition to any extent.

As a result of these findings, the action of the adrenotropic extract already described in normal rats can be attributed tentatively to a combination of these two effects. However, the action of a more purified adrenotropic extract upon both normal and adrenalectomized rats must be studied before this explanation can be considered final.

In the experiments with a sample of the "specific metabolic hormone," extract 622 of Collip and co-workers (secured through the courtesy of Doctor Collip), no effect on oxygen uptake or the R.Q. was observed. There was a profound reduction in the glucose absorption rate and apparently considerable glycogenolysis in the muscle, for there was no deposition of muscle glycogen. No influence on glycogen deposition could therefore be detected. The extract also appeared to have no glycostatic activity in hypophysectomized rats.

The action of A.P.E. in maintaining fasting muscle glycogen levels—the glycostatic action—seems still to be independent of the cortical hormone. It is active in hypophysectomized-adrenalectomized rats (17), and the glycostatic activity of A.P.E. bears no relation to the content of adreno-

tropic factor as determined by morphologic tests (2). The fasting muscle glycogen levels of adrenalectomized rats maintained on salt, while often somewhat low, especially in young animals, is still much above that found in hypophysectomized rats, as shown in table 1 and noted by others (5, 8). Adrenal cortical therapy sufficient to maintain normal levels in adrenalectomized rats is without effect in hypophysectomized animals

TABLE 2

Carbohydrate levels of rats fed glucose

	NUMBER OF RATS	GLUCOSE ABSORP. RATE	BLOOD SUGAR	MUSCLE GLYC.	LIVER GLYC.	OXYGEN CONSUMP.	R.Q.	NON-PROTEIN R.Q.
		mgm. per 100 gm. per hour	*mgm. per cent*	*mgm. per cent*	*per cent*	*ml. per 100 gm. per hour*		
A. Normal rats								
1. Untreated	19	185 ±3.2	127 ± 4.9	748 ±18	2.98 ±0.08	145 ±1.9	0.857 ±0.005	0.863
2. A.P.E.* (saline)	7	154 ±6.2	126 ± 7.9	860 ±51	2.58 ±0.33	168 ±6.0	0.754 ±0.004	0.747
3. A.P.E. (alkaline ext.)	13	173 ±6.2	142 ± 6.7	871 ±27	2.76 ±0.16	166 ±3.1	0.781 ±0.008	0.779
4. A.P.E.—adrenotropic	4	160	182	905	2.28	159	0.731	0.728
5. A.P.E.—Sp. Metabolic Hormone, 622	4	116	123	495	1.87	123	0.851	
6. C.E.†—corticosterone	8	191 ±6.1	155 ±10	834 ±41	3.11 ±0.23	153 ±2.5	0.811 ±0.008	0.812
7. C.E.—(Upjohn's)‡	10	171 ±5	154 ± 9	824 ±32	4.86 ±0.24	157 ±5	0.781 ±0.005	0.776
B. Adrenalectomized rats								
1. Untreated	11	143 ±8.1	121 ± 4.7	551 ±25	1.43 ±0.06	144 ±9.1	0.837 ±0.009	0.843
2. A.P.E. (saline)	10	115 ±4.0	134 ± 1.2	562 ±12	0.55 ±0.17	126 ±4.0	0.833 ±0.009	0.837
3. C.E.—0.5 ml	8	97 ±4.6	100 ± 3.0	485 ±16	1.54 ±0.26	119 ±5.7	0.818 ±0.010	0.821
4. C.E.—3-5 ml.	10	125 ±7.1	141 ± 3.9	566 ±20	2.04 ±0.31	137 ±6.3	0.800 ±0.003	0.800
5. C.E. (0.5 ml.) with A.P.E. (saline)	9	108 ±4.1	121 ± 5.6	612 ±17	1.17 ±0.19	134 ±3.3	0.775 ±0.005	0.772
6. C.E. (3-5 ml.) with A.P.E. (saline)	9	117 ±3.3	110 ± 3.2	761 ±17	1.74 ±0.21	152 ±6.1	0.767 ±0.006	0.762

* Anterior pituitary extract.
† Adrenal cortical extract.
‡ From B. Katzin and C. N. H. Long.

(2, 6, 10). Now, in addition, it appears that A.P.E. is capable of preventing any fall in muscle glycogen in adrenalectomized rats, without at the same time affecting the liver glycogen. The blood sugar level of these animals is decreased by the treatment (table 1).

The adrenal cortex, on the other hand, seems to be concerned with maintaining liver glycogen and blood sugar levels. The effects of adrenalectomy on these levels are closely similar to those of hypophysectomy.

The administration of adrenotropic extracts to hypophysectomized rats for a period of time stimulates the adrenal cortex effectively; liver glycogen and blood sugar levels are maintained above the normal in such animals (3). Then, C.E. given in large amounts to hypophysectomized rats causes large increases in liver glycogen, and C.E. can also increase the liver glycogen and blood sugar levels of normal rats and mice (12). The muscle

TABLE 3

Disposition of absorbed carbohydrate in rats fed glucose

		GLUCOSE RECOVERED IN			GLUCOSE OXIDIZED	TOTAL RECOVERED
		Body fluids	Muscle glycogen	Liver glycogen		
						per cent of absorbed glucose
A. Normal rats						
1. Untreated	mgm.	27 ±3	114 ±12	121 ± 4.2	370 ± 4	
	per cent*	3 ±0.3	16 ± 2	16.5 ± 0.7	49.5 ± 1.3	86
2. A.P.E.† (saline)	mgm.	28 ±5	165 ±26	106 ±15	113 ±17	
	per cent	5 ±0.9	27 ± 4	17.2 ± 2.4	18.3 ± 2.4	68
3. Cortical extract						
a. Corticosterone	mgm.	42 ±4	155 ± 7	136 ±10	267 ±30	
	per cent	6 ±0.6	20 ± 1	17.8 ± 1.7	35.1 ± 3.9	79
b. Cortical extract	mgm.	35 ±5	159 ±20	171 ± 8	154 ±13	
	per cent	6 ±0.7	23 ± 2	25.0 ± 0.7	25.7 ± 2.0	76
4. A.P.E. (adrenotropic)	mgm.	55	190	102	56	
	per cent	9	30	16	9	64
B. Adrenalectomized rats						
1. Untreated	mgm.	34 ±3	60 ±15	48.9 ± 5.6	314 ±29	
	per cent	6 ±0.6	10 ± 3	8.8 ± 1.0	56.3 ± 4.5	80
2. A.P.E. (saline)	mgm.	40 ±6	65 ± 9	22.0 ± 7.1	252 ±18	
	per cent	9 ±1.3	14 ± 2	4.8 ± 1.1	54.7 ± 6.0	83
3. Cortical extract						
a. 0.5 ml.	mgm.	23 ±2	28 ±12	55.9 ± 9.2	211 ±24	
	per cent	6 ±0.6	7 ± 3	15.7 ± 2.7	53.6 ± 6.0	83
b. 3-5 ml.	mgm.	45 ±3	65 ±12	80.0 ±14.2	206 ±22	
	per cent	9 ±0.5	13 ± 2	16.0 ± 1.4	40.6 ± 2.7	79
4. A.P.E. (saline) with C.E.						
a. 0.5 ml. C.E.	mgm.	33 ±3	92 ±11	43.9 ± 8.1	138 ±15	
	per cent	8 ±0.8	21 ± 2.5	10.2 ± 2.0	31.9 ± 3.9	71
b. 3-5 ml. C.E	mgm.	28 ±2	165 ±11	62.5 ± 7.8	135 ±13	
	per cent	6 ±0.5	35 ± 2	13.4 ± 1.4	29.1 ± 2.9	83

* Per cent of absorbed glucose.

† Anterior pituitary extract.

glycogen is not directly affected by cortical hormone administration. Finally, the effect of C.E. is additive to that of whole A.P.E. in hypophysectomized rats, the former affecting liver glycogen and blood sugar levels and the latter the muscle glycogen, as would be expected from the observations quoted above (18).

The problem of carbohydrate metabolism in adrenalectomized rats is

in some confusion owing to the inadequate attention paid by many investigators to the salt and food intake of these animals and to the frequent use of animals in the terminal stages of adrenal insufficiency, when physiological changes, only indirectly the results of adrenalectomy, may influence the results. When adrenalectomized rats are kept in good condition with adequate salt therapy and their appetites are well maintained, their carbohydrate levels before fasting are entirely normal. This finding has been noted by others (8, 19), and is shown in table 1. When they are fasted, however, there is a rapid fall in liver glycogen and blood sugar levels, as has been reported often. The muscle glycogen levels at the end of 24 hours may be normal or somewhat low (5, 8). This diminution in carbohydrate reserves may well be due to diminished gluconeogenesis from protein; for the nitrogen excretion of fasted adrenalectomized rats is about 30 per cent below normal (8, 11), and a number of other observations indicate diminished gluconeogenesis from protein in the absence of the adrenal cortex (13).

Although a complete study of the exact disposition of fed carbohydrate by adrenalectomized rats has not been made under optimal conditions, this process does not appear at present to be grossly abnormal. Several reports of normal absorption of glucose from the intestinal tract in adrenalectomized rats adequately treated with salt have been made (Althausen, et al., (1); Deuel, et al., (7)). In the experiments reported here, this rate was rather low, although not as low as in untreated hypophysectomized rats. The large amounts of hypertonic solutions given, poorly tolerated by the adrenalectomized rats, and the younger age of these animals probably accounts for this discrepancy. The only considerable change observed here in the disposition of the fed carbohydrate was a moderate decrease in the deposition of liver glycogen, with small increases in the proportions oxidized and remaining in the tissue fluids. These results are in contrast to those of Britton and Corey (4), who failed to observe any deposition of glycogen in adrenalectomized cats fed carbohydrate. They may also be contrasted with the disposition in hypophysectomized rats, in which most of the carbohydrate appears to be oxidized and little left for deposition. Normal liver glycogen deposition was obtained by Deuel et al. (7) in adult adrenalectomized rats treated with salt.

It is reasonable to conclude that the effects of A.P.E. and of C.E. in fasted animals, at least, are produced by two different mechanisms. A considerable weight of evidence has accumulated indicating that the effects of the adrenal cortical hormone are best explained as a stimulation of gluconeogenesis, probably from body protein (11, 12, 13). The A.P.E., however, seems ordinarily to have an anabolic rather than a catabolic effect on protein metabolism. Its effects on carbohydrate levels are explicable as direct effects on the oxidation of carbohydrate. In their

action on the disposition of fed carbohydrate, another relationship comes into play, namely, a synergism, as shown in these experiments. Further evidence of synergism appears in the recent work of Fry, Long, and Ritter (9), in which the glucose excretion of partially depancreatized-adrenalectomized rats maintained at a constant level with C.E. was further increased by the administration of A.P.E. This action can be explained as a diminution of carbohydrate oxidation superimposed on increased gluconeogenesis due to the presence of C.E., but made possible also only by the presence of some cortical hormone, as in the experiments described above. The question remains whether the C.E. directly affects only gluconeogenesis and glycogen storage, or whether, like A.P.E., it has also a primary effect on oxidation; for although it appears to depress oxidation when given in large amounts, the accumulation of glycogen in the livers rather than in the muscles suggests the possibility of its action being different in its mechanism from that of the A.P.E.

SUMMARY

In normal rats fed glucose, both whole anterior pituitary extracts and adrenal cortical hormone (when given in large amounts) appear to depress the utilization of carbohydrate and to promote the deposition of glycogen; the former increases muscle glycogen storage only, and the latter more markedly affects the liver stores. An adrenotropic extract of A.P.E., which also contained the metabolic factors, also depressed the oxidation of carbohydrate and increased muscle glycogen deposition.

In adrenalectomized rats, anterior pituitary extracts fail to act as in the normal rats. Large amounts of adrenal cortical hormone increased liver glycogen at the expense of oxidation, as in normal rats, but it did not affect muscle glycogen. When, however, the A.P.E. was given in the presence of adrenal cortical hormone, the usual action of the A.P.E. on oxidation and on muscle glycogen was fully developed. Since this effect was observed when the dose of cortical hormone was too small to be effective by itself, it was concluded that the A.P.E. and the cortical hormone have a synergistic as well as in part complementary action on the metabolism of fed glucose in rats.

In fasted rats, A.P.E. maintains muscle glycogen levels in the absence of the adrenal cortex, without affecting the liver glycogen or blood sugar levels, whereas cortical hormone, probably by promoting gluconeogenesis, increases liver glycogen stores and maintains blood sugar levels but does not seem to affect muscle glycogen stores directly.

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THE PRESSOR EFFECT OF KIDNEY EXTRACTS OF INTACT AND PARTIALLY NEPHRECTOMIZED RATS[1]

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In animals, experimental hypertension has been produced most consistently by renal ischemia (1) and by partial nephrectomy (2). Harrison and co-workers (3) and Prinzmetal, Friedman and Abramson (4) concluded that the injection of saline extracts prepared from the ischemic kidneys elevated the blood pressure of test animals to a greater extent than did similarly prepared extracts of control kidneys. The present paper describes the results of similar assays carried out with extracts made from the residual kidney tissue of animals rendered hypertensive by subtotal nephrectomy. It appears that there is no relationship between the hypertension of the partially nephrectomized rat and the amount of pressor substance in the kidney.

METHODS. Partial nephrectomy was performed in sixty day old rats of Wistar strain according to the procedure of Chanutin and Ferris (2); eighty to ninety per cent of the total renal tissue was removed. These animals were fed a stock diet (Bal Ra)[3] and were sacrificed after a minimum period of six months. At this time all the animals had tremendously enlarged arteries and cardiac hypertrophy which indicated marked hypertension (2). The rats were anesthetized with ether, exsanguinated by withdrawal of blood from the abdominal aorta and the kidneys were removed. The kidneys of intact rats served as controls. The kidneys were sectioned longitudinally, the medullary structures cut away, the tissue weighed and then ground in a mortar with glass. The macerated tissue was extracted with two per cent sodium chloride in a ratio of 1.5 cc. for one gram of residual kidney tissue and 2 cc. for one gram of normal tissue. The smaller amount of saline was used in the former case to compensate for the edema that is always present in these kidneys. The saline-kidney mixture was shaken for thirty minutes and was allowed to stand overnight at 6°C. After centrifugation, the supernatant fluid was adjusted

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[2] Nemours Foundation Fellow.

[3] Bal Ra, purchased from Valentine Meat Juice Company, Richmond, Virginia.

to pH 4.5 and the resulting protein precipitate was removed by further centrifugation. The supernatant extract was used for injection.

The test animals were mature male rats weighing between 250 and 350 grams. These animals were anesthetized by peritoneal injection of about 4 mgm. of sodium pentobarbital (Lilly) per 100 grams body weight. A needle connected by a three-way stopcock to a mercury manometer was introduced into the right carotid artery. Immediately after needling, 1 mgm. of heparin dissolved in 0.1 cc. saline was injected. The kidney extract was injected after the blood pressure was constant over a period of several minutes. The procedure was standardized by injecting 0.2 cc. of extract during a ten-second interval. Readings were taken immediately after injection and every fifteen seconds during the first minute, after every thirty seconds during the second minute and

TABLE 1

Effects of injection of saline extracts of the kidneys of intact and partially nephrectomized rats on the blood pressure of rats

Maximum rise of blood pressure in millimeters of mercury

RAT NUMBER	PARTIAL NEPHRECTOMY	RAT NUMBER	INTACT
1	10	10	34
2	8	11	28
3	8	12	32
4	8	13	36
5	10	14	24
6	0	15	28
7	14	16	36
8	8	17	24
9	4	18	26
Average.....	7.7		29.8

every sixty seconds during a total period of ten minutes. A different test rat was used for each determination.

RESULTS. The data showing the maximum increases in blood pressure after the injection of saline extracts of the kidneys of intact and partially nephrectomized rats into test rats are shown in table 1.

The results show that the pressor effects of the extracts from normal kidneys are consistently and definitely greater than those of extracts prepared from the kidney residues of the partially nephrectomized rats. In some cases the increase in blood pressure in the hypertensive kidney extracts is no greater than that obtained by injecting physiological or two per cent sodium chloride. A preliminary depressor effect was absent in practically all cases.

A number of procedures were used for preparing kidney extracts before

adopting the procedure described above. Crude saline extracts, extracts clarified with the ultracentrifuge and extracts heated to 55–56°C. (5) yielded the same type of result as that obtained by bringing the pH to 4.5.

An attempt was made to eliminate extraneous factors by fractional precipitation of the active globulin with varying concentrations of sodium sulfate. It was found that this procedure did not affect the type of pressor response obtained with the respective extracts.

COMMENT. In view of the results obtained in these experiments, it appears that the kidneys of subtotally nephrectomized rats contain little or no pressor substance. These findings differ from those obtained with the ischemic (Goldblatt) kidney of the dog. It might be postulated that renin cannot be stored in the kidneys of the partially nephrectomized rats because of the pathological alterations, and it is therefore being excreted or poured into the blood stream. Our efforts to demonstrate a pressor material in the blood or urine of these hypertensive rats have yielded negative results.

SUMMARY

Saline extracts of the kidney remnant of hypertensive, partially nephrectomized rats produced little or no pressor effect when injected into normal rats. This is in marked contrast to the consistent and striking pressor effect obtained with extracts of normal rat kidneys.

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RADIOACTIVE IODINE AS AN INDICATOR IN THYROID PHYSIOLOGY

Iodine Collection by Normal and Hyperplastic Thyroids in Rabbits[1]

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In previous papers (1, 2) we have described the techniques and advantages of the use of radioactive isotopes of iodine as indicators in the study of iodine distribution and have reported preliminary results obtained with this method. This paper is concerned with the detailed results of extended experiments on normal rabbits and on rabbits which had received previous treatment designed to influence the physiologic state of the thyroid.

The majority of the experiments were conducted with the use of the radioactive isotope of iodine of mass 128, which has a half period of 25 minutes. We have also used a few samples of radioactive iodine with the half periods of 12.5 hours, 8 days and 13 days (mass numbers 130, 131 and 126 respectively) (3).

Purpose of experiments. It was the purpose of these experiments to investigate the collection of iodine by normal and hyperplastic thyroid glands, in order to establish the normal and pathological behavior toward iodine under various circumstances, and in order to determine the conditions under which it might be possible to use radioactive iodine to administer internal irradiation of the thyroid. The experiments have therefore been concerned with the measurement of the percentage collection of known doses of labelled iodine by the thyroid, as a function of time of collection, quantity of iodine injected, previous history of iodine treatment, thyrotropic hormone administration, cyanide injection, cabbage diet, sex, pregnancy, and certain combinations of these factors.

Procedures. The iodine was usually administered intravenously in the form of sodium iodide obtained by dissolving labelled silver iodide in sodium thiosulphate (2). In a few experiments in which long collection times were possible because of the availability of long-period iodine iso-

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topes, subcutaneous injections were made. Animals were sacrificed by etherization, since the available radioactivities necessitated the removal of the thyroid for measurements.

The dosages of iodine administered (except in some preliminary work) were adjusted to be proportional to the weight of the animal, 2 kgm. being taken as a standard. Dosages below 0.5 mgm. were not so adjusted, however, it being of interest to determine the collection of the smallest available quantities of iodine. The range of dosages used was from less than 0.1 mgm. to 100 mgm.

In the present experiments, in addition to the information given by the gross appearance of the gland, we have used as a rough measure of the physiologic state of the thyroid the relative weight of the thyroid as compared with the body weight. It is well known that this is by no means a completely satisfactory indication of the physiologic state of the gland, but nevertheless, with a standard stimulus and large numbers of animals, it was possible to obtain statistically significant results.

For these purposes we have defined a quantity we call the thyroid index, as follows:

$$\text{Thyroid index (T.I.)} = \frac{\text{Weight of thyroid in milligrams}}{\text{Body weight in kilograms, } \times 10}$$

where the factor of 10 is introduced to give a convenient numerical result. For the normal animals in our colonies, the thyroid index of almost all animals ranged between 5.5 and 8.0. (See fig. 1.) In hyperplastic animals the index ordinarily ranges between 8.0 and 15.0, the highest value observed in our colony being 33.5 for an animal on an exclusive cabbage diet for 2 months.

In measuring thyroid iodine collection, we have introduced a quantity we call the thyroid concentration coefficient, in order to take into account the variation of size of thyroid glands among animals of the same weight. This quantity is defined by the relation

$$\text{Thyroid concentration coefficient (T.C.C.)} = \frac{\text{Thyroid iodine collection in per cent, } \times 1000}{\text{Thyroid index}}$$

The introduction of the factor 1000 has the result of making this quantity represent the concentration of iodine in the thyroid divided by the concentration in an equal weight of body tissue, assuming uniform distribution of the iodine. (Thus it is a measure of the affinity of the thyroid for iodine.)

This method of expressing the iodine collected by the thyroid is particularly useful because it gives directly the concentrating power of the

thyroid as compared with the rest of the body. In some preliminary work to determine the approximate character of the iodine collection of various stimulated glands, no indices were constructed, and the data are accordingly presented in terms of percentage. A direct comparison between percentage collection and thyroid concentration coefficient may be obtained from figures 4 and 6. Previously published data (1) have shown that the collection of iodine by the thyroid is much greater than by other organs.

All animals (except those on a cabbage diet) were maintained on a uniform rabbit chow diet, in a room kept free of any gross contamination with iodine. Thyrotropic hormone, cyanide and iodine other than the

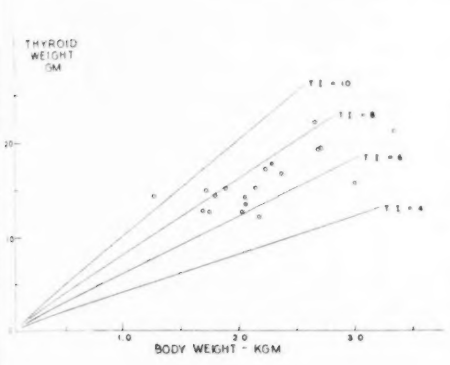

Fig. 1

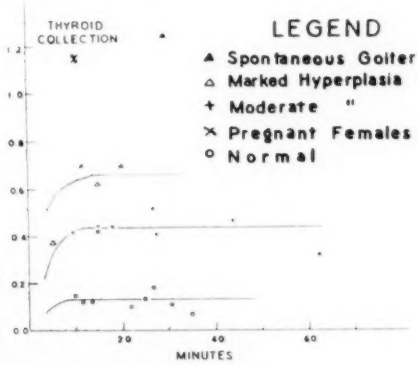

Fig. 2

Fig. 1. The variation of thyroid weight with body weight for some of the normal animals used. The radial lines are lines of constant thyroid index.

Fig. 2. Preliminary survey of the percentage collection of the thyroid in various time intervals after injection of a 10 mgm. dose of iodine. The data show the marked dependence upon the functional state of the thyroid. No precautions were taken to adjust the size of injection to the size of the animal, and the degree of hyperplasia was judged from the appearance of the gland alone.

labelled iodine were injected subcutaneously or intramuscularly. Animals on a cabbage diet were fed exclusively on cabbage, no water being given.

Cyanide-injected animals were maintained on a normal diet, and were given daily injections of 0.1 cc. of methyl cyanide daily for 2 to 4 weeks. Animals that were given 0.25 cc. daily exhibited a very high mortality rate without a great change in thyroid response.

Several different preparations of thyrotropic hormone have been used during the course of the experiments. These include suspensions of fresh beef anterior pituitary, of desiccated anterior pituitary powder supplied by Armour and Co., material prepared according to the technique of

Lambie and Trikojus (4) (in both the soluble and insoluble forms), and a thyrotropic extract prepared and kindly furnished to us by Dr. Oliver Kamm of Parke, Davis and Company. Of all these the last named was found to be the most active and satisfactory preparation. With this material 50 to 100 per cent increase of thyroid size in normal adult male rabbits may be obtained with 2 successive daily injections, corresponding to a nominal daily dose of 25 guinea-pig units. In early experiments, Ayerst, McKenna and Harrison thyrotropic preparations were used, but were found to be inactive in male rabbits, in dosages up to 10 cc.

Pretreatment with iodine. A series of experiments were undertaken to determine the effect upon the collection of a labelled dose of iodine of

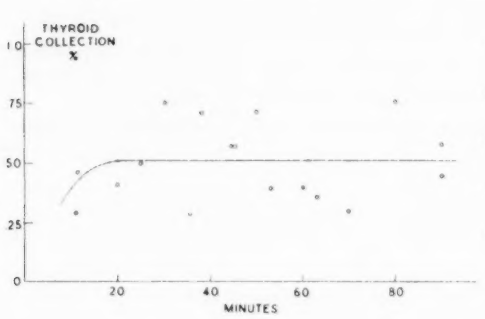

Fig. 3

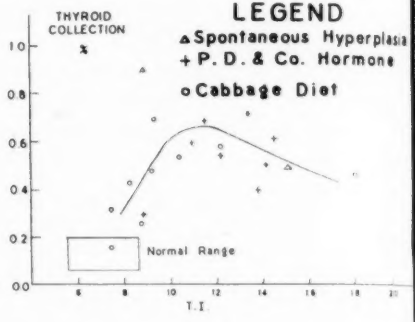

Fig. 4

Fig. 3. Percentage collection of the thyroid of normal males of injections of between 0.1 and 0.4 mgm. of iodine. No increase of collection over that of the first 10 minutes is observed for intervals up to an hour and a half.

Fig. 4. Percentage collection of hyperplastic thyroid glands of male animals. Hyperplasia induced by cabbage diet, by injection with Parke, Davis and Company thyrotropic hormone, and of spontaneous occurrence. Injection 5 mgm. per standard 2 kgm. animal. Collection time 15 minutes. The differently treated animals show experimentally indistinguishable responses, with a maximum collection at T. I. ca. 11 or 12. These animals are referred to as type 1.

previous iodine treatment. In these experiments the labelled dose of iodine was always 5 mgm., while the amount used in pretreatment was varied from 5 mgm. to 100. The time elapsing between pretreatment and injection of the labelled dose of iodine was varied from several minutes to several days.

Experiments with more than one radioactive isotope. In some of these experiments it was possible to label the dose used for pretreatment as well as the succeeding dose, using a different radioactive isotope for each dose, and distinguishing between the 2 or more radioactivities found in the thyroid by following the decay curve of the total radioactivity in the

gland, and analyzing it into its several components. In the analogy of labelling, this corresponds to the use of labels of different colors for the different doses.

RESULTS. Most of our data are presented in graphical form, in figures 1 to 10.

Using the long-period isotopes referred to above, we have obtained some data on the collection of single doses of iodine in hyperplastic thyroids in periods up to 8 days. The results show that the collection in type 1 animals (see figs. 4–6 for explanation of different types) tends to increase until the total collection has reached the value of about 40 to 50 micrograms, and then does not increase further. The length of time this takes

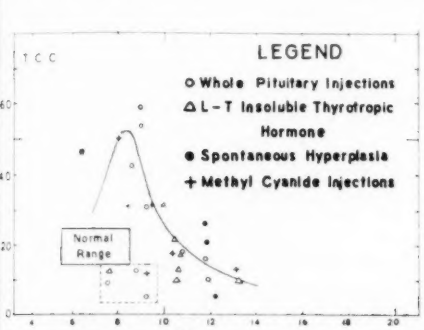

Fig. 5

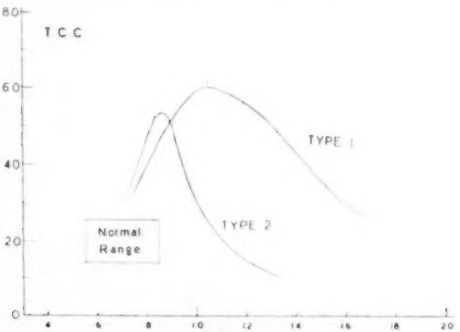

Fig. 6

Fig. 5. Thyroid collection coefficients of male animals treated as follows: saline suspensions of fresh beef anterior pituitary, insoluble thyrotropic hormone prepared according to the method of Lambie and Trikojus, methyl cyanide injected, and untreated animals with spontaneous hyperplasia. Injection 5 mgm. per standard 2-kgm. animal. Collection time 15 minutes. For a discussion of the points in the dotted rectangle, which were not taken into account in drawing the curve, see the section entitled "Discussion." These animals are referred to as type 2.

Fig. 6. Thyroid concentration coefficients for the data of figures 4 and 5.

depends mainly upon the total quantity of iodine injected. From figure 9 this may occur in as little as 15 minutes. The smallest quantities of long-period labelled iodine injected have been of the order of 1.0 to 1.5 mgm. The thyroid collection of this quantity may go as high as 6 per cent within a day, in animals on a cabbage diet. This is to be compared with collections of as much as 6 per cent within 15 minutes with injections of ca. 0.2 mgm.

In an extension of the results given in figure 10, 6 animals have been injected with 2 successive labelled doses of iodine and the collection from each dose measured. The results seem to show that if a moderately large collection occurs from the first dose, the collection of the second dose is

inhibited. Thus an animal which in 19 hours collected 53 micrograms from a 4 mgm. injection collected less than 4 micrograms from the subsequent 5 mgm. injection. On the other hand, an animal which had collected only 20 micrograms from a 5.5 mgm. injection in 31 hours, collected 7 micrograms from the subsequent 5 mgm. injection. The collection time of the second injection was always 15 minutes. In one animal whose collection time was 8 days, the collection was not much more than it would have been in 15 minutes.

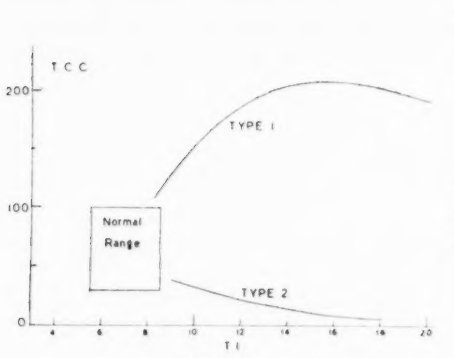

Fig. 7

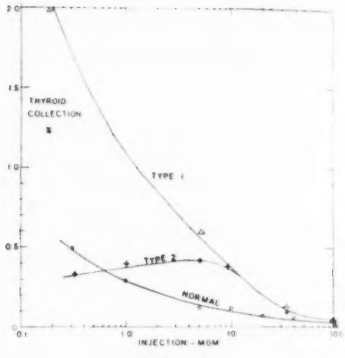

Fig. 8

Fig. 7. Same type of data as figure 6 for injections of 0.2 to 0.5 mgm. per standard 2-kgm. animal. In type 2 animals are now included some which received a dilute acetic acid suspension of Armour's desiccated pituitary tablets, and some receiving the soluble thyrotropic hormone prepared according to the method of Lambie and Trikojus (4). The curve for type 1 animals is only approximate in character, since the scattering of the values of the thyroid collection coefficient is great, the values ranging from 100 to 500. The number of animals used was 12 type 1, 20 type 2.

Fig. 8. Variation of the percentage iodine collection of the thyroid in 15 minutes with the quantity of iodine injected. The curves for hyperplastic animals are for average thyroid index 10. Total number of animals used: 39 type 1, 68 type 2, 69 normal.

In most of the experiments on pretreatment with iodine, the premedication was accomplished with ordinary iodine. The effects of premedication depend upon a large number of other factors in animals with hyperplastic thyroids. The subsequent collection appears to depend not only upon the quantity of the premedicating dose, but also upon the thyroid index of the animals, the type of thyroid stimulation, and to some extent the time elapsing between the premedicating dose and the labelled dose. In normal animals a dose of about 50 mgm. is necessary to reduce the collection of the labelled dose to half the value expected of a normal untreated animal. In the case of type 1 animals, only 8 mgm. will do the same within 4 to 6 hours. (See fig. 10.)

A number of control experiments with radioactive bromine have been performed. The thyroid collection in both normal and hyperplastic animals is less than one-tenth of that from the same quantity of iodine, and the presence of the bromine could be demonstrated in the cerebrum within 15 minutes, in contra-distinction to the behaviour of the iodine, which could not be detected there.

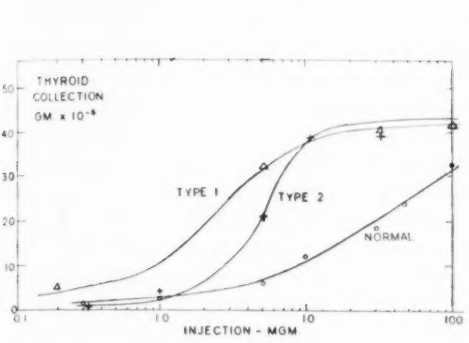

Fig. 9

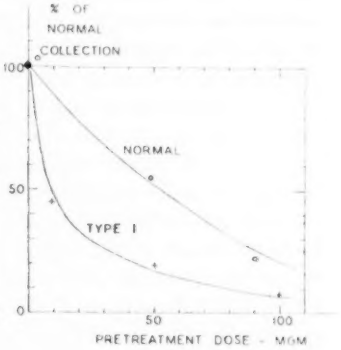

Fig. 10

Fig. 9. Total iodine collection of the thyroid from different injections in 15 minutes. The data are the same as those plotted in figure 8.

Fig. 10. The effect of iodine premedication upon the collection of a subsequent 5 mgm. labelled iodine injection in 15 minutes. The collection is given in terms of the collection without previous treatment, which is set at 100 per cent. The time elapsing between the two doses is between one hour and five days, in normal animals. The curve for type 1 animals is for thyroid index 10, and for an interval of time between the premedicating dose and the labelled dose of 4 to 6 hours. The number of animals used was 9 normal, 12 type 1.

Discussion of results. From figures 2 and 3 we see that the collection of iodine in both the normal and hyperplastic gland reaches a value within 10 minutes which is not thereafter exceeded for at least 1.5 hours. This has been verified for all the values of injection for which experimental points are plotted in figure 8. In a few cases, collection times of less than 5 minutes have been used; collection has been detected in animals that died within 2 minutes of injection. It appears, therefore that the *initial collection* (i.e., the collection within 1.5 hours) *is taken up in a time determined mainly by the time required for the injected material to reach the thyroid.* In the few experiments performed with long-period iodine, we have found that in general the increase of collection, if any, is comparatively slow following the initial collection.

The percentage of iodine collected from any given injection by the thyroid is definitely dependent upon the dosage, in all types of thyroids.

Figure 8 shows this dependence. For normal thyroids, *the percentage collection increases as the dosage is decreased.* The total quantity of iodine so collected, however, is small even when the injected dose is 10 mgm. or more. On the other hand, the efficiency of collecting a dose of 0.1 mgm. is much higher (see fig. 8). In terms of the concentration coefficient, the T.C.C. at 0.1 mgm. is about 80 for normal glands, while for an injection of 100 mgm. it may be as low as 2, or less. This relatively large percentage uptake from small injections seems qualitatively consistent with the ability of the normal gland to maintain itself upon the small quantities of iodine normally found in the diet.

From figures 4, 5, and 6, it appears that under various conditions of stimulation the thyroid can respond in (at least) two different ways, with respect to the iodine collection of doses of 5 mgm. In one type, which we have called type 1, the T.C.C. is always higher than normal, and in the other, type 2, the T.C.C. is significantly lower, and falls below normal for large values of the T.I. (In fact, for several of the animals—those in the dotted rectangle in fig. 5—the T.C.C. is below normal although the gland has increased only slightly in size. This will be discussed a little later on.) The two different types are defined by the character of their pretreatment, except in the case of animals with spontaneous hyperplasia, which were found to fall in both categories. There is for each type a maximum T.C.C. at a particular value of T.I. It is quite striking that glands of the same size, e.g., T.I. 13, do not exhibit the same iodine collection, although their gross appearances are similar, both appearing hyperplastic. The decrease of T.C.C. for thyroid indices larger than 10 can be interpreted in different ways. If we admit that the uptake of iodine under the conditions stated is a measure of the functional activity of the thyroid, then this decrease may be interpreted as due either to the existence of a threshold, or to functional exhaustion of the gland (or both). If we assume it to be due to a threshold, then we must suppose that in general the higher the thyroid index in type 2 animals, the higher the threshold for iodine uptake.

In this case we should expect the shape of the curves for type 2 animals to change, as the dosage of iodine is changed. Figure 7 shows the altered shape of the curves for very small injections. In this case, the type 1 animals follow the same sort of curve as in figures 4 and 6, and exhibit very high collections, reaching in certain cases as much as 6 per cent of the injection in 15 minutes. On the other hand, the type 2 animals always collect less than the normal at this injection level. This curve casts some light upon the animals referred to above, represented by the points in the dotted rectangle in figure 5.

These animals have thyroid indices only slightly greater than normal, but their collections are below normal even with a 5 mgm. injection. A

similar curve at an injection of 10 mgm. does not show any such points. Therefore, we have drawn the curve in figure 5 through the upper points, considering these animals to have a threshold for iodine collection which is greater than the average at their thyroid index. In some of the animals which received very small injections, the collection of iodine by the thyroid was so small that it was undetectable (less than 0.05–0.1 per cent of the injection) and only maximum values for the iodine collection could be given. The contrast between the type 1 and type 2 animals is even more striking at this value of injection than at the higher injection value.

Figures 8 and 9 again show the threshold effect, which can best be discussed with reference to the latter. In this graph we see that the total iodine collected by type 2 glands of a given size (T.I. 10) is rather high and fairly constant for injections above 10 mgm., but that it drops sharply between 1 and 10 mgm., becoming consistently lower than the normal collection somewhere near 1 mgm. On comparison with the behavior of the type 1 animals it appears that this is quite definitely a threshold effect and perhaps even an "all-or-none" effect. The relatively gradual decrease in total iodine collection of the type 1 animals shows that if similar considerations apply to these glands, they do not begin to do so in the dosage range investigated.

The functional exhaustion involved in the type 2 animals is not a complete exhaustion, but a threshold effect, because at injections of 10 mgm. and higher values, the collection of these animals is above normal in all cases, and is indistinguishable from the behavior of the type 1 animals.

It is interesting to note that for large doses, the collection of iodine in both types of glands attains a nearly constant value at about 40 to 50 micrograms. If the wet weight of a rabbit thyroid is about 200 mgm., of which 60 per cent is water, this quantity of iodine represents a collection of 0.05 per cent of the dry weight, or a quantity of the same order of magnitude as the original iodine content of a hyperplastic gland. Thus the hyperplastic gland is capable of increasing its iodine content 100 per cent within a few minutes.

We do not wish to suggest that the two types of hyperplastic glands discussed here constitute strictly defined separate categories. The curves of figure 9 seem to indicate that perhaps there can be continuous gradations of all types between the limits plotted in the graph, and even outside these limits. Evidence for this is given by the shape of the curves giving the variation of collection with thyroid index, which indicate that the threshold for iodine collection is somewhat displaced for values of the thyroid index other than 10. It has, of course, been previously known that various types of thyroid hyperplasia exist which are functionally different. The dissimilarity in behavior toward iodine is another indication of this multiplicity.

We have so far assumed that the appearance of the labelled iodine in the thyroid after injection is actually due to addition of new iodine to the gland, and not to simple exchange of the iodine previously present with the labelled iodine. We have several reasons for supposing this to be the case, although even if it were not, the iodine exchange could be used as an indication of the thyroid function, or to introduce internal irradiation, just as well as iodine collection. Our reasons for believing that we are dealing with collection rather than exchange are the following: hyperplastic glands collect more iodine than normal glands at some injection values, less at others. The amount exchanged by a gland ought ultimately to be independent of dosage, and to depend mainly upon the iodine content of the gland. This does not appear to be the case. Furthermore, the collection of the second dose of iodine is almost always much less than the collection of the first dose. The amount exchanged ought to be independent of whether the dose is the first or any other in order.

In view of the fact that there is a difference in iodine collection at the same thyroid index, at low injection values, in animals treated with Parke-Davis thyrotropic hormone and animals treated with other thyrotropic hormone preparations, including the saline suspension of fresh beef anterior pituitary, it appears that there may be separate factors producing growth and functional stimulation with respect to iodine collection, respectively. This is in accord with the clinical observation that the size of the thyroid, taken by itself, is not indicative of the functional state.

The difference between the behavior of cabbage diet animals and animals injected with methyl cyanide is worth noting, in view of the supposed cyanide nature of the goitrogenic substance in cabbage (5).

Radiation therapy with radioactive iodine. From the data on iodine collection that we have obtained, and the known energy of the radiations from the various radioactive isotopes of iodine, it is possible to calculate how strong the radioactivity of an administered dose of radioactive iodine must be in order to give any desired amount of radiation within the thyroid. Since the thyroid concentration coefficient may be as much as several hundred, it is easy to see that the ratio of thyroid dosage to the dosage of other tissues may be made just as high, thus giving a very large safety factor for such irradiations.

If we assume that it is desired to administer such an amount of radioactive iodine as to yield, in decaying to zero activity, a dose of 100 r within the thyroid, then the amount of initial activity required per gram of thyroid tissue has been calculated (6) to be:

For 25 min I: 100 r = 0.1 millicurie per gram thyroid
For 8 day I: 100 r = 0.6 microcurie per gram thyroid.

These activities are very different from each other, and we must now consider the Bunsen-Roscoe reciprocity law. It is a question still under

debate as to whether a weak source supplying radiation for a long time will have the same effect as a strong source supplying the same total radiation. The initial rates of irradiation for the above activities are respectively:

25 min. I : 2.7 r. per minute
8 day I : 0.4 r. per hour

Normal tissues tolerate safely a dosage of 0.1 r. per day for indefinite periods; it is therefore questionable whether the 8-day iodine of the strength calculated above is supplying its radiation fast enough to have the desired effects. This is something to be decided by experiment. On the other hand, the short-period iodine will approximate quite well the short intense dosage obtained in x-ray therapy.

Considering only the 25-minute period, then, the necessary activity per gram of tissue is 0.1 mC., for an initial intensity of ca. 3 r. per minute. If we assume a thyroid to collect 1 per cent of the administered iodine, as the results of these experiments permit us to suppose likely, the injected dosage must then be 10 mC. per gram of tissue. For the rabbit, with a thyroid of ca. 0.2 gram, we must correct the value thus obtained for the "leakage" of radiation outside the thyroid tissue, since some beta rays can escape from the thyroid because of its relatively small size, and will dissipate part of their energy in the surrounding tissue. This will certainly be taken care of by adding 50 per cent to the required dosage. Thus, for the rabbit, we may calculate that the required dosage to provide 100 r. of radiation in the thyroid is about 3 mC. of the 25-minute isotope of iodine.

Since preliminary experiments show that similar collections may be obtained in human thyroids, then a 75 gram thyroid will require an administered dosage of ca. 750 mC.

Acknowledgments. The experiments with the long-period isotopes of iodine were made possible through the generosity of Prof. E. O. Lawrence and Dr. Joseph Hamilton of the University of California, and Dean L. A. DuBridge of the University of Rochester, who supplied these isotopes from their cyclotron laboratories.

We are indebted to Dr. Gregory Pincus and Dr. Mark Graubard of Clark University for their kind assistance in maintaining animals on a cabbage diet, and for administering cyanide injections. We are particularly grateful to Dr. Oliver Kamm, of Parke, Davis and Company, who supplied us with an active thyrotropic hormone preparation.

SUMMARY

Using radioactive iodine as an indicator, the quantity of iodine taken up by the thyroid of the rabbit under various circumstances was studied. After intravenous injection, the percentage collection from any given dose was found to reach a maximum within ten minutes, which was not greatly

exceeded for periods of collection as long as several days. The normal thyroid was found to collect up to 80 times the quantity to be expected from uniform diffusion into the general body tissues; the hyperplastic thyroid will collect up to several hundred times the quantity expected from uniform diffusion. The variation of this concentration with the injected dosage and the functional state of the gland was determined. The effect of pretreatment of the thyroid in various functional states, with iodine, on the collection of a subsequent dose of labelled iodine was measured.

In certain of these experiments, several differently labelled iodine injections were used on the same animal in order to determine the fate of the individual doses. This labelling was accomplished by using different radioactive isotopes.

From the data thus obtained, we have calculated the strength of samples of radioactive iodine, with which it will be possible to administer internal irradiation of the thyroid for therapeutic purposes.

Clinical implications of the results are discussed.

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THE RELATIONSHIP BETWEEN THE ADRENAL AND PARATHYROID GLANDS

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A functional relationship between the adrenal and parathyroid glands has been suggested by Schour and Rogoff (1, 2), based, in part, upon the observation of disturbances in the calcification of dentin of the incisors of adrenalectomized rats similar to those produced by injection of parathyroid hormone, as reported by Schour, Tweedy and McJunkin (3). In addition, Rogoff and Stewart (4) noted a hypercalcemia in adrenalectomized dogs and Rogoff (5) found the parathyroid glands grossly enlarged.

Little has been written which bears directly on this problem. Long ago Eppinger, Falta and Rudinger (6) postulated an antagonistic relationship between parathyroids and adrenals. Grollman (7), in his monograph on the adrenals, and Shelling (8), writing on the parathyroids, conclude that valid evidence of a relationship is lacking. Perla and Sandberg (9) found changes in the incisors of adrenalectomized rats mainly unlike those described by Schour and Rogoff. Pugsley and Collip (10) reported that adrenalectomy augmented the effects of parathormone injection. Hoskins and Bernstein (11) could find no changes in the adrenal cortex lipoids of parathyroidectomized rats.

The lack of agreement of the few available reports led us to study the concentration of serum-calcium and the morphology of the parathyroids in adrenalectomized rats and to investigate the effects of treatment with cortical extract and sodium chloride.

MATERIALS AND METHODS. Twenty-two sets of male albino rats derived from the Wistar strain were used at an age of 10 weeks. Of the 4 animals of each set, litter-mates when possible, one was adrenalectomized and received no additional treatment; a second was adrenalectomized and given subcutaneous injections of eschatin; the third was adrenalectomized and given an option of 2 per cent aqueous solution of sodium chloride or ordinary tap water to drink; the fourth was subjected to a dummy operation and served as control. They were segregated in wire-bottom cages according to treatment received, furnished with an ample supply of tap water and fed the stock diet (whole ground wheat, 67.5 per cent; casein, 15 per cent; whole milk powder, 10 per cent; unsalted butter, 5.2 per cent; calcium

carbonate, 1.5 per cent; sodium chloride, 0.8 per cent; and cod liver oil in an amount equivalent to 1 per cent by weight of the preceding). All rats used had grown at an average rate and appeared healthy.

Under ether anesthesia and with antiseptic precautions bilateral adrenalectomy was performed in one stage through lumbar incisions. Each rat was weighed daily. Those given eschatin (adrenal cortex extract prepared by Parke, Davis and Co., said to contain at least 25 dog units per cc.) received subcutaneous injections daily or every other day. The amount of sodium chloride solution consumed in each cage was measured daily.

Each set of rats was killed when death appeared imminent in the untreated adrenalectomized member. Following anesthetization with ether body weight and length were measured, the thorax was opened widely and blood was withdrawn from the right ventricle of the heart by means of needle and syringe. The parathyroids and adjacent structures were removed and fixed in Zenker's fluid. Finally, the lungs were examined for pneumonia, the peritoneum for inflammation and the abdominal cavity for incomplete removal of the adrenals or adrenal rests. The blood was treated in the manner prescribed by the Clark-Collip modification of the Tisdall method for the determination of serum-calcium. The parathyroids and contiguous structures were imbedded in paraffin, sectioned at 8 microns, mounted serially and stained with Harris' hematoxylin and eosin Y. The volume of the parathyroids was measured by the paper outline method (12).

As an aid to interpreting results standard biometric formulae (13) were used. A measure of the likelihood that an observed difference may be due to chance is obtained from the ratio of the difference between means to its probable error. When this value, the significance ratio, is 3.0 or more, it means there is less than 1 chance in 20 that the observed difference is accidental. Accordingly, differences with a significance ratio of 3.0 or more have been interpreted as significant.

Criteria of uniformity of the various groups of animals were initial body weight and final body length, assuming that length was not affected during the short course of the experiment. Data given in table 1 show that all groups were essentially alike in these respects.

RESULTS. *General data.* Autopsy was performed at an average of 7 days after adrenalectomy, when death appeared imminent in the untreated adrenalectomized member of each group. In none of the adrenalectomized rats was adrenal remnant or accessory adrenal tissue grossly visible. Two had pneumonia.

The untreated adrenalectomized rats were weak, cold and had sunken eyeballs. They lost weight rapidly the first 3 days, slowly thereafter. At autopsy they weighed 14.3 per cent less than the controls, which was a significant loss (table 1).

The eschatin-treated adrenalectomized rats received a mean daily dose of 0.35 cc. and an average total dose of 2.34 cc., which, according to avail-

TABLE 1

Statistical data

GROUP	NUMBER	MEAN ± S.D.	DIFF. ± P.E.	S.R.	PER CENT VALUES
Initial body weight—grams					
Controls	21	209.7±24.0			
Untreated tests	22	207.3±27.1	−2.4±5.3	0.5	98.85
Eschatin treated tests	22	207.7±23.7	−2.0±4.9	0.4	99.04
Sodium treated tests	21	207.9±21.8	−1.8±4.8	0.4	99.14
Final body weight—grams					
Controls	21	218.5±25.0			
Untreated tests	22	187.3±24.8	−31.2±5.1	6.1	85.72
Eschatin treated tests	22	200.7±30.3	−17.8±5.7	3.1	91.85
Sodium treated tests	21	210.9±24.4	−7.6±5.2	1.5	96.52
Body length—centimeters					
Controls	21	20.18±0.82			
Untreated tests	22	19.72±0.80	−0.46±0.17	2.8	97.72
Eschatin treated tests	22	20.08±0.77	−0.10±0.16	0.6	99.50
Sodium treated tests	21	20.10±0.60	−0.08±0.15	0.5	99.60
Parathyroid volume—cubic millimeters					
Controls	18	0.1853±0.0350			
Untreated tests	18	0.1776±0.0280	−0.0077±0.0071	1.1	95.84
Eschatin treated tests	16	0.1965±0.0210	0.0112±0.0066	1.7	106.04
Sodium treated tests	19	0.1945±0.0237	0.0092±0.0067	1.4	104.90
Serum calcium level—milligrams per 100 cc.					
Controls	17	11.84±2.00			
Untreated tests	17	10.88±1.73	−0.96±0.42	2.3	91.89
Eschatin treated tests	18	11.86±1.73	0.02±0.42	0.1	100.17
Sodium treated tests	18	10.78±1.41	−1.06±0.38	2.8	91.04

S.D., standard deviation; P.E., probable error; S.R., significance ratio.

able information, amounted to at least 9 dog units daily. These animals were neither weak nor cold, but the mean final body weight, 8.2 per cent less than that of the controls, represented a significant loss (table 1).

The sodium-treated adrenalectomized rats consumed optionally a daily average of 17.3 cc. of 2 per cent aqueous sodium chloride solution, were neither weak nor cold and showed an insignificant mean loss of body weight amounting to 3.5 per cent (table 1).

Parathyroid morphology. Mean values and other data on volume are given in table 1. Compared with the control group the mean parathyroid volume of the untreated adrenalectomized rats was 4.2 per cent less; of the eschatin-treated group, 6.0 per cent greater; and of the sodium-treated animals, 4.9 per cent greater. None of these changes was statistically significant. However, the change in size of the parathyroids from the intermediate control value was consistent with the treatment received. The mean parathyroid volume of the untreated adrenalectomized rats was 9.6 per cent less than that of the eschatin-treated group and 8.7 per cent less than that of the sodium-treated group; and both differences were statistically significant. Microscopic examination showed no histologic differences.

Serum-calcium. Mean values and statistical data are given in table 1. The mean serum-calcium level of the control group was 11.84 mgm. per 100 cc.; of the untreated adrenalectomized rats, 8.1 per cent less, probably significant; of the eschatin-treated animals, 0.2 per cent more, insignificant; and of the sodium-treated group, 9.0 per cent less, probably significant.

Discussion. Any inferences drawn from the collected data as to an adrenal-parathyroid relationship rest upon the common assumption that parathyroid size and serum-calcium level are indicators of the degree of parathyroid activity. On this basis, for which we make no claims, our data showed that bilateral adrenalectomy did not lead to hyperactivity of the parathyroid glands in male albino rats. On the contrary, our findings of diminution in volume of the parathyroids and lowering of the serum-calcium level point in the opposite direction, viz., that bilateral adrenalectomy resulted in hypoactivity of the parathyroids. Although control and test values did not differ significantly, certain additional considerations lend support to this surmise.

The untreated adrenalectomized rats lost most body weight and had the smallest parathyroids. Was the parathyroid change part of the general trend? The observation of Jackson (14) that parathyroids did not diminish in size in underfed rats, and our finding the largest parathyroids in eschatin-treated rats, which nevertheless had lost body weight to a significant degree, are against such an interpretation. The parathyroids of the untreated adrenalectomized rats were significantly smaller than those of the eschatin and sodium-treated animals. These changes, in opposite directions from the intermediate control value and consistent with the experimental procedures, indicate a synergistic rather than an antagonistic relationship. Because the administration of sodium chloride had the same

effect as eschatin it is suggested that the changes in the parathyroids were mediated through effects on sodium metabolism.

Consideration of serum-calcium changes must include certain qualitative observations. Large amounts of blood were drawn readily from the sodium-treated animals, moderate amounts from the control and eschatin-treated rats; but only small samples could be obtained from the untreated adrenalectomized rats. On centrifuging, the yield of serum was least from the blood of untreated and most from the blood of sodium-treated adrenalectomized rats. The mean control serum-calcium value was 11.84 mgm. per 100 cc.; that of the untreated adrenalectomized rats, 10.88 mgm. If serum volume is diminished in the latter, then total serum-calcium must be significantly reduced. Nor can this be associated with the loss of body weight, for the eschatin-treated rats, which also lost body weight to a significant degree, had a mean serum-calcium level of 11.86 mgm. The low serum-calcium level of the sodium-treated animals may have been the result of an hydremia. The lack of quantitative data on blood volume and plasma-cell proportions render a conclusion difficult, but the ease with which large samples of blood were withdrawn from the heart and serum was obtained by centrifuging make reasonable such an explanation. Thus, adrenalectomy was followed by a drop in serum calcium which was probably significant. Injection of eschatin prevented this drop. Administration of sodium chloride apparently did not prevent the drop but its effect may have been masked by hydremia.

SUMMARY AND CONCLUSIONS

To investigate the existence of an adrenal-parathyroid relationship, changes in serum-calcium level and parathyroid volume after adrenalectomy were studied in 10 week old male albino rats. Of each of 22 sets of 4 animals one was adrenalectomized, a second adrenalectomized and injected with eschatin, a third adrenalectomized and given an option of 2 per cent aqueous solution of sodium chloride or ordinary tap water to drink, and a fourth, the control, subjected to a dummy operation. The animals were killed at an average of 7 days after operation, when death appeared imminent in the untreated adrenalectomized rats.

Adrenalectomy was followed by a decrease in size of the parathyroids not statistically significant and by a drop in serum-calcium level of probable significance. Treatment with eschatin prevented these changes and caused the parathyroids to become significantly larger than those of the untreated adrenalectomized rats. Treatment with sodium chloride solution had the same effect as eschatin on parathyroid size but did not prevent the drop in serum-calcium level. The latter may be apparent only, because of hydremia.

The data compiled show that adrenalectomy did not lead to hyper-

activity of the parathyroids; that either there is no functional relationship or that adrenalectomy led to hypoactivity of the parathyroids, possibly because of the effect on sodium metabolism. The concurrence of diminished parathyroid volume and lowered serum-calcium level in adrenalectomized rats, and of increased parathyroid volume and normal serum-calcium level in eschatin-treated rats supports the last mentioned view.

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THE AMOUNT OF ASCORBIC ACID EXCRETED AT EACH URINATION DURING TWENTY-FOUR-HOUR PERIODS[1,2]

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Many studies have been made to determine the state of vitamin C nutrition in human beings by determining the urinary output of ascorbic acid. Some workers (1, 2) have used 24-hour samples of urine for their tests, while others (3, 4, 5) have suggested that the ascorbic acid content of urine samples collected during a specified portion of the day would indicate the nutritional state as far as vitamin C is concerned.

Owing to lack of agreement as to the number of hours that should be included in a daily test period and the time of day during which the collections should be made, there seemed to be a need for more information about the amount of ascorbic acid excreted at each urination during the twenty-four hours, over a period of days. For such a study it appeared advisable to use individuals whose normal diet showed a relatively high content of ascorbic acid.

PROCEDURE. Two women, engaged in similar occupations, were secured as experimental subjects. Their physical characteristics were as follows: Subject A, age, 37 yrs.; height, 5 ft. 8 in.; weight, 130 lbs. (59.1 kgm.): Subject B, age, 51 yrs.; height, 5 ft. 5 in.; weight, 175 lbs. (79.5 kgm.).

These subjects were requested to make complete collections at each urination and keep them separate. For convenience, the 24-hour period was arbitrarily set up as extending from 7 a.m. one morning to 7 a.m. the next morning. According to this plan, the first morning urination, upon rising, was always included in the previous day's records. The test period extended over ten days.

Following each urination, the volume of urine was carefully measured and a 25 cc. portion was drawn off and added to an equal volume of 6 per cent metaphosphoric acid, according to the directions of Bessey (6). Usually this sample was tested for its ascorbic acid content soon after

[1] This investigation is part of the regional project of the Northwest States on the ascorbic acid metabolism of college students.

[2] Paper no. 131, Montana Agricultural Experiment Station. Approved by Director.

collection, with the exception of the sample obtained at 10 o'clock in the evening. Some unpublished results from work done in this laboratory indicate little, if any, loss of ascorbic acid in urine samples held over night in this 3 per cent metaphosphoric acid mixture.

TABLE 1

Three-day records for two subjects showing amount of ascorbic acid (A-A) excreted at each urination during 24-hour periods

TIME OF EXCRETION	1ST TEST DAY		2ND TEST DAY		3RD TEST DAY		MEAN OF THREE TESTS	
	Volume of urine	Ascorbic acid	Volume of urine	Ascorbic acid	Volume of urine	Ascorbic acid	Volume of urine	Ascorbic acid
Subject A								
	cc.	*mgm.*	*cc.*	*mgm.*	*cc.*	*mgm.*	*cc.*	*mgm.*
8 a.m.	333	5.8	163	8.2	525	16.0	340	10.0
11 a.m.	730	37.4	645	35.1	618	44.3	664	38.9
1 p.m.	185	13.9	278	17.7	275	33.6	246	21.7
4 p.m.	490	39.3	245	38.2	154	29.2	296	35.6
10 p.m.	425	35.4	337	24.1	370	24.3	377	27.9
7 a.m.	285	27.4	212	14.2	480	12.1	326	17.9
24-hour excretion....	2,345	149.9	1,880	137.8	2,422	159.5	2,216	149.6
Percentage A-A excreted in 6 hrs., 7 a.m.–1 p.m.....		38.1		44.5		58.9		47.1
Subject B								
11 a.m.	207	28.4						
1 p.m.	69	12.6	273	21.3	230	14.9	191	16.3
4 p.m.					69	14.4		
10 p.m.	285	27.4	275	25.2	150	14.4	237	22.3
7 a.m.	400	36.8	400	11.2	350	27.8	383	25.3
24-hour excretion....	961	105.2	948	57.7	799	71.5	903	78.1
Percentage A-A excreted in 6 hrs., 7 a.m.–1 p.m.....		38.9		36.9		20.8		32.2

These urine samples were tested for the amount of ascorbic acid present by means of the Evelyn photoelectric colorimeter, using the method described by Bessey (6). The total amount of ascorbic acid excreted in each urination was then calculated, as well as the total amount for each 24-hour period.

During each day of the test period a careful record was kept of all food and liquid consumed. The liquid included water, coffee, tea and milk.

By consulting tables giving the vitamin C content of foods (7, 8), an estimate was made of the ascorbic acid intake per day.

RESULTS. The data obtained during three typical days of the test period are presented in table 1. This gives the daily record for each subject, including the volume of each urination, the milligrams of ascorbic acid excreted in each urination, and the totals for each day. Upon examining the data, it is readily seen that the volumes of the individual urinations for both subjects differ considerably and that the amounts of ascorbic acid excreted in each of the urinations vary widely. Also, there is no correlation between the volumes of the individual urinations and the amount of ascorbic acid contained in each.

TABLE 2

Ten-day records for two subjects showing comparison of ascorbic acid (A-A) intake and output during 24-hour periods

DAY OF TEST	SUBJECT A					SUBJECT B				
	Intake		Output			Intake		Output		
	Estimated A-A intake per day	Liquid intake per day	Volume urine in 24 hours	A-A excreted in 24 hours	Percentage A-A intake excreted	Estimated A-A intake per day	Liquid intake per day	Volume urine in 24 hours	A-A excreted in 24 hours	Percentage A-A intake excreted
	mgm.	*cc.*	*cc.*	*mgm.*	*per cent*	*mgm.*	*cc.*	*cc.*	*mgm.*	*per cent*
1	262	830	2,345	149.9	57.2	123	1,019	961	105.1	85.4
2	228	1,778	1,880	137.8	60.4	110	640	948	57.7	52.4
3	126	2,133	2,422	159.5	126.5	143	877	799	71.5	50.0
4	190	2,963	2,723	134.1	70.5	145	948	1,048	97.4	67.1
5	255	2,678	1,422	303.3	118.9	153	948	1,095	111.4	72.8
6	292	2,536	2,062	301.1	103.1	87	1,019	980	63.8	73.3
7	211	2,062	1,773	154.9	73.4	230	1,019	685	101.1	43.9
8	156	1,967	2,182	180.2	115.5	133	830	832	119.9	90.1
9	194	1,541	2,528	171.4	88.3	27?	1,067	1,185	152.0	55.6
10	234	2,133	2,415	105.9	45.2	133	711	645	72.0	54.1
Average....	215	2,062	2,174	179.8	83.6	153	908	918	95.2	62.2

Since some workers (3, 4, 5) have suggested the use of a urine collection period shorter than 24 hours, and since it has been reported (3) that the maximum return of a massive dose of ascorbic acid comes between the 3rd and the 6th hours, a six-hour collection period has been adopted in certain studies. The nutrition workers, who are coöperating in the northwest regional project, studying the ascorbic acid metabolism of college students, have used the six-hour period from 7 a.m. to 1 p.m. during the past year.

For this reason, the percentage of the total ascorbic acid excretion occurring in this six-hour period is recorded in table 1. In three days, sub-

ject A excreted an average of 47.1 per cent during these six hours, while in the ten-day test period, the average excretion was 41.6 per cent (range 14.5–63.5). Subject B excreted an average of 32.2 per cent during the six hours for the three typical days, and 33.9 per cent (range 15.3–47.6) for the ten-day period. In view of the wide variation noted in the values obtained from this six-hour collection period, its use for determining "resting level" might be questioned.

Some additional data are presented in table 2, including the ascorbic acid intake per day as estimated on the basis of food records kept by each subject. The amount of daily liquid intake, and the total output of urine and ascorbic acid for each 24-hour period are noted. For the purpose of comparing the daily output of ascorbic acid with the estimated intake, the percentage of output was calculated. It is interesting to note that in a few cases this percentage of output is more than 100 per cent.

The average data for the 10-day period were further interpreted, using Sendroy's (9) utilization coefficient and index.

$$\frac{\text{Intake ascorbic acid—output ascorbic acid}}{\text{Body weight in kgm.}} = \text{utilization coefficient}$$

$$\text{Utilization coefficient} \times \sqrt{\text{age}} = \text{utilization index}$$

	AVERAGE DAILY INTAKE A-A MINUS OUTPUT A-A	BODY WEIGHT	UTILIZATION COEFFICIENT	$\sqrt{\text{AGE}}$	UTILIZATION INDEX
	mgm.	*kgm.*			
Subject A	35.2	59.1	0.597	6.08	3.6
Subject B	57.8	79.5	0.727	7.15	5.2

Small indices such as those given above, indicate that both subjects were in a good state of vitamin C nutrition according to Sendroy (9).

SUMMARY

The volume of urine and the amount of ascorbic acid excreted at each urination varied widely throughout the 24-hour period. There is no correlation between the volume of each urination and its ascorbic acid content.

When the amounts of ascorbic acid excreted during 6-hour periods, from 7 a.m. to 1 p.m., are compared with the total amounts of ascorbic acid excreted during the complete 24-hour periods, the variation in results is great enough to question the use of this 6-hour collection period for determining "resting level."

The daily intake of ascorbic acid was estimated for each subject and compared with her ascorbic acid output for that 24-hour period.

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SYMPATHETIC ACTION IN ACCOMMODATION FOR FAR VISION

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It has been shown that a sudden exciting stimulus causes a brief change in the refraction of the rabbit's eye (Olmsted and Morgan, 1939a) in the direction of increased hypermetropia and in the human eye as well (Olmsted and Morgan, 1939b). It was suggested that this reaction is the result of sympathetic stimulation. Further experiments now show that the sympathetic may indeed play a major role in the processes involved in accommodation for far vision and a minor one in that for near vision.

a. *Sympathetic nerve stimulation.* The eyes of one series of 4 rabbits were refracted by the usual method of skiascopy as described in a previous paper (Olmsted and Morgan, 1939a). The average value for all readings on the 8 eyes when the rabbits were held quietly was +3.08 D. Under ether anesthesia the left sympathetic nerve of each rabbit was exposed and arranged for stimulation with a faradic current by means of a Harvard inductorium. Etherization was then maintained lightly and the nerve stimulated. Because of the accompanying extreme pupillary dilatation, the investigation was limited to the central area of the cornea and lens by means of an artificial pupil, 4 mm. in diameter. The average value for the 4 eyes during sympathetic nerve stimulation was +4.17 D, an increase in the direction of hypermetropia of +1.00 D. At this same time the average value for the 4 right eyes was +3.10 D, i.e., at the stage of anesthesia at which the sympathetic was stimulated on one side, the refractive state of the other eye was the same as that of the unanesthetized, unexcited rabbit.

This experiment was repeated with another series of 6 rabbits with similar results. Here the stimulation was maintained for at least 5 sec., and the increase in hypermetropia during stimulation was in some cases as high as +2.50 D, the average increase being +1.66 D.

There can be no doubt that in the rabbit, at least, stimulation of the sympathetic nerve causes flattening of the lens to a marked degree. This had already been claimed to be the case as early as 1888 by Jessop. Morat and Doyon (1891), Terrien and Camus (1902), Byrnes (1933), and Cogan (1937) confirmed these results, but Trautvetter (1866), Langley and An-

derson (1892), and Römer and Dufour (1902) failed to do so. Hess and Heine (1898) found that stimulation of the sympathetic in the dog did increase hypermetropia from +1.00 D to +1.50 D, but explained their results as being due to dilatation of the pupil which, in their opinion, allowed the periphery of the cornea and lens to influence skiascopic findings. Scepticism regarding the influence of the sympathetic on accommodation of the lens has at present gone so far that in practically none of the current diagrams showing the innervation of the ciliary muscles is there pictured any sympathetic connection with these muscles, but only IIIrd nerve connections.

If this latter were the case, flattening of the lens would have to be brought about solely through central inhibition of tonic impulses over the oculomotor nerve. But it is evident that such can not be the mechanism involved when the sympathetic is stimulated peripherally and the eye becomes more hypermetropic. The action here is a peripheral (motor) one, not central (reflex), as is shown by the fact that it occurs on stimulating the peripheral end of the *cut* sympathetic.

b. *Stimulation of the sympathetic with the IIIrd nerve cut and vice versa.* To show the reciprocal relations of the sympathetic and oculomotor nerves, the following experiments were performed: The eyes of a cat were refracted before administering ether. The results were R.E., +1.00 D; L.E., +1.00 D. Under light ether the refraction of R.E. changed to −0.50 D, that is, the eye became more myopic. The left superior cervical ganglion was then stimulated with a faradic current. The eye became extremely hypermetropic, but the refractive condition did not remain constant during the stimulation, varying between +4.00 D and +6.00 D. Because the pupil dilated so strongly an artificial pupil was used. The skull of the cat was now trephined, the IIIrd nerve exposed at its exit from the brain stem and cut as close to the brain as possible. The pupil became extremely dilated, and again an artificial pupil was used for refraction. The left eye gave +3.50 D, i.e., was strongly hypermetropic. The left superior cervical ganglion was immediately stimulated and the refraction increased to +10.00 D. The sympathetic nerve was then cut and the refraction returned to −0.50 D.

Exactly similar, but rather less striking, results were obtained on two other cats. A similar experiment was performed on a dog. Under ether both eyes gave −0.50 D. When the whole left vagus-sympathetic complex was stimulated refraction changed to +1.00 D, an increase in the direction of hypermetropia of +1.50 D. The left IIIrd nerve was then exposed and cut, but there was no change in refraction. Stimulation of the vagus-sympathetic, however, again increased hypermetropia to about +2.00 D. This could not be determined accurately because of the appearance of so-called "scissors-motion."

In another cat, whose normal refraction was L.E., +1.50 D; R.E., +1.50 D, 2 per cent atropine was instilled into the eyes. In half an hour paralysis of IIIrd nerve endings was complete, as shown by failure of the pupil to respond to light flashed into the eye. When the superior cervical ganglion was stimulated there was an increase in hypermetropia of +1.00 D.

The superior cervical ganglion in this cat was removed aseptically. One week later both eyes were refracted: L.E., +0.50 D; R.E., +2.00 D. The eye with its sympathetic nerve supply gone had become more myopic. The left IIIrd nerve was now exposed and stimulated. The result was a further increase in myopia to the extent of −2.00 D to −3.00 D.

These experiments show that stimulation of the sympathetic after paralysis of the IIIrd nerve by atropine or cutting results in a change in the lens in the direction of hypermetropia. Likewise after removal of the superior cervical ganglion stimulation of the IIIrd nerve causes myopia.

c. *The extrinsic eye muscles.* Objection might be made that these results in some measure might be due to pressure on the eyeball of the extrinsic muscles. This objection would be practically ruled out by the experiments where the IIIrd nerve was cut. But to obtain definite information on this point a rabbit was decorticated under ether so that subsequent operations might be performed with the minimum administration of anesthetic. All the extrinsic muscles to the left eye were severed, except the external rectus, and the retractor bulbi. These muscles were left to afford some support for the eyeball, but any pressure exerted on the eyeball by them would be too irregular to be effective in changing accommodation of the lens. Refraction of L.E. gave +2.50 D. The left IIIrd nerve was then exposed and stimulated. The eye became myopic to the extent of 2.00 D. The left IIIrd nerve was now cut and an hour later the reading was +3.00 D. The left sympathetic was then stimulated and refraction went to +3.50 D. The experiment was repeated several times with the same results.

Two of the four retractor bulbi muscles were removed in order to disclose the short ciliary nerves. The ciliary ganglion was stimulated, with the result that the eye showed a refractive error of +0.50 D. This is to be expected since the short ciliary nerves contain fibers from the IIIrd nerve and sympathetic fibers as well. The short ciliary nerves were now destroyed. The refraction immediately after destruction of these nerves was still +0.50 D, probably because of irritation from the cut ends of the nerves. Stimulation of the sympathetic caused the refraction to change to +1.50 D. Therefore elimination of possible pressure effects from the extrinsic ocular muscles does not interfere with production of hypermetropia on stimulating the sympathetic or myopia on stimulating the oculomotor.

Since the sympathetic is able to produce hypermetropia in the absence of the oculomotor nerve, and the oculomotor to produce myopia in the absence of the superior cervical ganglion, the principle of reciprocal innervation is presumably operating in the processes concerned with the accommodation of the lens for far and near vision, just as it does in the iris where the same two nerves are involved (Gullberg, Olmsted and Wagman, 1938).

SUMMARY

1. Stimulation of the sympathetic nerve or superior cervical sympathetic ganglion in the rabbit, cat and dog gives marked increase in hypermetropia (flattening of the lens) whether the eye is atropinized, the IIIrd nerve is intact or cut, or whether pressure effects on the eyeball are eliminated by cutting the majority of the extrinsic muscles.

2. Removing the superior cervical sympathetic ganglion in the one cat so operated led to permanent decrease in hypermetropia in the corresponding eye.

3. Stimulation of the oculomotor nerve in the rabbit and cat gives marked myopia whether the sympathetic is intact or cut.

4. Accommodation of the eye for near and far vision presumably involves reciprocal action of the sympathetic and IIIrd nerves.

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SECRETION OF URINE IN THE CHICKEN (GALLUS DOMESTICUS)

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The collection of uncontaminated specimens of avian urine presents considerable difficulty because the urine of the bird flows from the ureters into the cloaca where it may mix freely with fecal matter. Previous investigators have devised a variety of methods for obtaining avian urine free of dejecta.

The observations on the quantity and character of the urine excreted by birds have usually been incidental to work on other problems concerned with avian renal physiology. To what extent variations in the method of collection influenced the quantity of urine excreted was not determined. Some workers made their study with the birds under general anesthesia while others used unanesthetized birds. In one report three species of fowl were used and the urine output stated as 600 to 1500 cc. in twenty-four hours without the author indicating whether these figures applied to each species or to the whole group. Some investigators obtained their data from observations made over a short time while others collected their data over an extended period. To obtain an adequate conception of the daily volume and character of the urine secreted by birds under a variety of conditions further work appeared necessary. Consequently a series of experiments done with several methods, some of which have been used by previous workers, forms the basis of the present report which deals only with urine secretion in the domestic chicken (Gallus domesticus).

METHODS. The methods followed in collecting avian urine have varied with the different investigators as the following summary of the literature indicates. Minkowski (1) circumvented fecal contamination of the urine of the goose by ligating the rectum above the cloaca which as Milroy (2) suggests may have militated against the accuracy of his observations. Milroy in an investigation of uric acid formation in ducks and geese attempted to obtain urine free of fecal matter by means of a specially devised tube designed to divert the feces from the portion of the apparatus that was to carry off the urine. Fecal contamination was not prevented. Milroy then separated the rectum and cloaca by an operation. An arti-

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ficial anus was made in the anterior abdominal wall and the urine was collected in a suitable bag fixed below the anal orifice for periods as long as twenty-four hours during which time the quantity passed varied from 500 to 1500 cc.

Paton (3) collected urine from geese, ducks and hens. An artificial anus was produced as in Milroy's experiments but the urine was collected in a metabolism cage. According to Paton's statement: "The amount of urine passed in twenty-four hours could not be accurately estimated since the birds spilt some of their drinking water into it. As observed it was between 600 and 1000 cc." Sharpe (4) working with hens under urethane or ether anesthesia obtained urine by cannulating the ureters. The calculated urine output for twenty-four hours was 500 to 1000 cc. Mayrs (5) cannulated the ureters of cockerels under urethane anesthesia. In an average of seven experiments the urine output was 0.69 cc. per minute. If this rate of flow were maintained, nearly 1000 cc. of urine would be excreted in twenty-four hours (our calculation).

Davis (6) studied the nitrogenous constituents of the urine of hens with and without ether anesthesia. Urine was collected by means of a catheter which was inserted into the urodeum in such a manner as to drain off the urine as it flowed from the ureteral orifices. To prevent fecal contamination cotton plugs were sometimes inserted in the rectum. Under ether anesthesia 55 to 60 cc. of dilute urine were passed in an hour. The author stated that, without anesthesia, "The urine was so concentrated that the solids precipitated and clogged the catheter." Because of this he gave water by mouth just before the collection of the urine and a maximum output of about 30 cc. per hour was obtained. Coulson and Hughes (7) in connection with their studies on the nutrition of chickens used the method of Davis for collecting urine in unanesthetized birds. They obtained from 33 to 70 cc. of urine in sixty minutes. The longest period of collection recorded was 100 minutes during which 48 cc. of urine were collected. Young and Dreyer (8), using the method of Sharpe, by calculation found the daily output of urine by the chicken to be 48 to 480 cc. They expressed the belief that the higher flows were owing to diuresis which, in their opinion, was caused by the anesthetic.

The chickens used in our experiments were hatched and raised on the farm of the laboratory. In all, six brown leghorns, four Jersey white giants and twenty white leghorns were used. They were mature male and female birds about one year of age, weighing at the beginning of the experiment from 1.1 to 3.3 kgm. Throughout the experiments they were maintained on the same ration which consisted of two parts of wheat, one part of oats and one part of cracked corn. The chickens had access to oyster shell grit and water at all times if the nature of the experiment permitted it. Each bird was kept in a separate cage.

Three series of experiments were done: 1. The urine was collected by cannulating the ureters under local anesthesia and the effect of certain diuretics and other drugs was observed. 2. The method of collecting the urine was similar to that used by Davis. A small funnel of sufficient diameter to include the two ureteral orifices was inserted into the urodeum and the urine collected was free of fecal matter. 3. The ureteral orifices were exteriorized by an operation which made possible the collection of urine under as nearly normal conditions as we could devise.

The pH of the urine was measured by a potentiometer. A more complete description of the procedure followed and the data obtained in experiments done with each method will be given with the record of the results.

RESULTS. 1. *Urine flow in cannulated ureters, effect of drugs.* It had been found in previous work that general anesthesia increased the quantity of urine secreted (Davis); therefore, in a series of ten chickens the ureters were exposed and cannulated under local infiltration anesthesia. Procaine in a 1 per cent solution was injected into the skin and subcutaneous tissues along the course of the ureters in sufficient quantity to produce anesthesia. Three cubic centimeters were usually adequate. The exposure and cannulation of the ureters was easily accomplished after a little practice. Further anesthesia was unnecessary.

The chickens were placed in a box just large enough to allow them to stand comfortably. A canvas hammock, containing openings for the legs, supported the body. An opening in one end of the box permitted the head and neck to project while the other end was entirely open. Excessive movement could be further prevented by proper restraint of the wings. During the course of the experiments the birds usually remained quiet, at times falling asleep.

Ten experiments of this nature were done on ten different birds. Immediately after the ureters had been cannulated collection of the urine in graduated tubes was begun. To prevent loss of volume through evaporation the tubes were covered with a rubber membrane and the cannulas were inserted through a hole in the latter. The output at the end of a thirty-minute period was determined. The birds were then unmolested for thirty minutes, after which another thirty-minute sample was collected. In the ten experiments, with one exception, the output during the first thirty minutes after cannulation of the ureters was many times that of the second period. The character of the urine secreted bore a definite relation to the quantity. The first control thirty-minute sample which averaged 13.9 cc. for the ten experiments, was a clear, slightly greenish, watery fluid usually free of particulate matter and slightly acid to litmus. For the second period when the output was relatively small (average for 30 min. 2.5 cc.) the urine was a yellowish, thick, tenacious, mucoid material

containing large amounts of urates in clumps or in thread-like strands. The pH of urine with these characteristics was 6.3 to 6.7. The flow of urine was usually intermittent. It was repeatedly noted that when urine poured from one ureter the other was quiescent. In one series of observations alternation of ebb and flow in the two ureters was rhythmic with a periodicity of about ten seconds.

Following the control collections several agents were given intravenously and their effect on urine secretion determined in two successive periods of thirty minutes each. Caffeine and glucose were effective diuretics. Sodium sulfate and ephedrine did not produce appreciable diuresis in the doses used. Pituitrin definitely decreased urine flow in the one experiment

TABLE 1

Volume of urine collected from chickens for thirty-minute periods following cannulation of both ureters and following injection of certain diuretics and other drugs

NUMBER	SEX	WEIGHT	AFTER CANNULATION		DRUGS INJECTED INTRAVENOUSLY	AFTER INJECTION OF DRUGS	
			First specimen	Second specimen		First specimen	Second specimen
		kgm.	*cc.*	*cc.*		*cc.*	*cc.*
1	F	1.42	15.2	2.7	1 cc. of 1% caffeine sodium benzoate	10.4	3.0
2	M	2.20	19.2	1.5	50 cc. of 20% glucose	3.0	24.2
3	M		13.3	3.2	50 cc. of 20% glucose	48.3	40.7
4	M	2.60	18.2	3.0	1 cc. of 1% caffeine sodium benzoate	9.0	1.7
5	M	2.60	15.2	2.1	1 cc. of 4% sodium sulfate	2.8	2.7
6	F	1.17	12.5	2.8	1 cc. of 4% sodium sulfate	3.0	3.0
7	M		13.2	3.6	50 cc. of 20% glucose	42.5	31.6
8	F	1.43	12.4	2.2	Ephedrine 20 mgm.	2.4	
9	F	1.10	18.2	1.9	Ephedrine 20 mgm.	2.3	
10	F	1.27	1.3	1.9	Pituitrin 0.02 cc.	0.2	0.2
Average.....			13.9	2.5			

in which it was given. Only 0.2 cc. of urine was secreted in each of the periods of collection following the injection of 0.02 cc. of surgical pituitrin (table 1).

The results of the first series of experiments showed that large flows of urine occurred in the first control period but during the second the output was sharply curtailed, a result which agrees with the observations of Young and Dreyer. When the average flow for the first thirty-minute period of collection was calculated for twenty-four hours, the output was 667 cc. which is within the range of values reported by many previous workers. However, when the average flow for the second control period was so calculated the daily urine output was only 120 cc. It seemed possible that the operative procedures may have induced stimuli that resulted in an

increased flow of urine during the first period. The latter is not explained by general anesthesia as suggested by Young and Dreyer in experiments on anesthetized birds.

2. *Urine flow with the funnel technic.* To obviate the possible effects on urine flow of the operative procedures necessary in the foregoing experiments, another series of experiments was done which will be described in this section of this report. In this series of experiments a technic for collecting the urine was used similar to the one described by Davis and later by Coulson and Hughes. The chickens were placed in the boxes already described. The cloaca was emptied and washed free of feces. In a few experiments it was necessary to plug the rectum with cotton to prevent fecal contamination. A small funnel was then inserted into the urodeum. The urine was thus carried from the two ureteral orifices into graduated tubes. In the group of ten birds used in this series seven females and three males were included. All of the birds had been maintained under as nearly identical conditions as could be devised. They were brought repeatedly to the laboratory and two to four successive thirty-minute samples of urine were taken. This was done usually on consecutive days.

In the present series the average output of urine by the first four birds in the initial period of collection was 2 to 3.4 cc. and for the second period it was from 2.3 to 3.3 cc. which is within the range of values obtained from cannulated ureters during the second period of collection. Had more experiments not been done it might have been concluded, erroneously, that the operative procedures in the first series of experiments were responsible for the birds secreting a much larger volume of urine during the first period of collection. In every one of the next six birds the output during the first period of collection was many times that of the following periods. The average flow for the first period on four different days was from 9.8 to 13.4 cc. but the flows for the succeeding periods were similar to the values obtained with cannulated ureters in the second period of collection (table 2).

Judging from the data just presented it was evident that the presence of the funnel in the urodeum was not the factor responsible for the increased flow of urine during the first period of collection. In most of the experiments the chickens were brought to our laboratory from their cages in another building and immediately after arrival were prepared for observation. It was decided to determine whether this amount of disturbance could be a factor in producing an increased flow of urine in the majority of the birds during the first period of collection. To test this idea three hens were selected. They were brought to the laboratory on August 27, 28 and 30 (a.m.) and immediately thereafter on each occasion observations were made. Food and water were withheld for thirty minutes prior to

the time they were brought to the laboratory on the days just mentioned. The same birds were again brought to the laboratory on August 30 (p.m.) and 31 and on September 1 without food and water having been previously

TABLE 2

Volume in cubic centimeters of urine collected with funnel technic from chickens for successive thirty-minute periods on four different days

NUMBER	SEX	FIRST DAY	SECOND DAY	THIRD DAY	FOURTH DAY	AVERAGE
20	F	1.5 2.3	4.0 3.4	5.2 4.1	3.1 3.3	3.4 3.3
21	F	2.2 1.9	1.9 2.6	2.6 3.1	2.0 1.8	2.2 2.3
22	F	2.8 3.4	1.8 2.0	1.9 2.4	2.8 3.0	2.3 2.7
23	F	2.0 2.0	2.1 3.2	1.6 1.8	2.3 4.4	2.0 2.8
24	M	8.6 2.8 2.3	11.2 1.5 2.4	9.7 2.4 1.3	10.8 1.2 1.1	10.1 2.0 1.8
25	M	15.2 2.1 2.5	13.4 1.8 2.4	8.2 2.0 4.5	13.5 2.0 3.2	12.6 2.0 3.1
26	F	11.2 3.8 4.1 3.6	18.2 3.1 2.5	12.0 2.4 2.3	12.3 1.8 2.3	13.4 2.8 2.8
27	F	9.0 2.7 2.5	20.0 2.5 3.6	12.3 2.0 2.0	11.2 1.9 2.3	13.1 2.3 2.6
28	M	12.0 4.3 3.1	9.7 3.9 2.8	11.8 1.5 2.0	10.3 2.2 1.3	10.9 3.0 2.3
29	F	8.0 2.4 2.9	8.5 2.0 2.9	13.3 2.3 2.5	9.6 3.0 2.4	9.8 2.4 2.7

withheld. They were allowed to remain ready for observation but were undisturbed for thirty minutes when their urine output was again measured for the next thirty minutes. Food and water were at all times available to them.

In every case when the urine was collected just after the arrival of the birds in the laboratory the flow was high, the volume varying in the three birds from 10 to 17.2 cc. with an average of 12.3 cc. but the volume of urine secreted on the days the birds had remained undisturbed in the laboratory for thirty minutes was small, ranging from 1.2 to 5 cc. The average for the group was 2.7 cc. (table 3). The mechanism responsible for the difference in the output of urine observed, under the conditions just described, was not determined but it seems evident that variations in the food and water intake were not responsible for the results. These experiments serve to illustrate the difficulty of arriving at a reliable estimate of the

TABLE 3

Volume in cubic centimeters of urine secreted for thirty-minute periods on the dates indicated; urine collected with funnel technic

NUMBER	SEX	DATE	NORMAL*	DATE	NORMAL†
12	F	Aug. 27	11.0	Aug. 30 p.m.	1.2
		Aug. 28	13.3	Aug. 31	3.4
		Aug. 30 a.m.	12.0	Sept. 1	5.0
19	F	Aug. 27	12.0	Aug. 30 p.m.	1.3
		Aug. 28	10.8	Aug. 31	1.8
		Aug. 30 a.m.	10.0	Sept. 1	4.3
30	F	Aug. 27	17.2	Aug. 30 p.m.	2.4
		Aug. 28	13.2	Aug. 31	2.0
		Aug. 30 a.m.	11.0	Sept. 1	2.8
Average			12.3		2.7

* Chickens were brought into the laboratory and collections begun immediately. Food and water had been withheld for thirty minutes just prior to their being brought to the laboratory.

† Chickens were brought into the laboratory and were undisturbed for thirty minutes before beginning collection of urine. Food and water were available to them at all times.

normal urine output of the chicken by the use of two methods previously employed by other workers.

As a result of the foregoing experiments it is obvious that accurate information on the daily output of urine by the chicken cannot be obtained by calculations from flows obtained for a short time. Further experiments were devised and will now be described.

3. *Urine flow from exteriorized ureteral orifices.* For this series of experiments the ureters of seven chickens were exteriorized under general anesthesia produced by the intravenous administration of 25 mgm. of pentobarbital sodium (nembutal) per kilogram of body weight. A rectangular flap of skin was freed between the dorsal border of the anal

margin and the ventral surface of the pygostyle. The anal margins including the anal sphincter were incised midway on each side. The dorsal portion of the anal ring was then pulled upward and a continuous skin suture line was formed, attaching the dorsal portion of the anal ring to the ventral surface of the pygostyle. The mucosa lining the dorsal portion of the cloaca was then separated from the muscular layer of the bowel wall. After careful dissection of the mucosa it was incised approximately 5 mm. anterior to the ureteral openings which were readily identified owing to the character of urine being voided. The urine was passed to the exterior and in all seven chickens the bowels moved normally. When healing had occurred small funnels made of gauze covered with collodion were sutured in place so as to drain off the urine as it flowed from the two ureteral orifices. This group of birds had been accustomed for a period of two weeks to wear a harness which would support a rubber balloon attached to the tapered end of the funnel for the purpose of collecting the urine.

The operative procedures in this method of collecting avian urine were considered less drastic than those used by Milroy and others and they could likewise be produced with less difficulty. This method also had the advantage of making possible the collection of the urine directly from the ureters. The possible mixture of secretions from the mucosa of the cloaca or from the oviducts of the hens was prevented.

When the chickens were ready for observation the rubber bags were attached to the funnels, after which the birds went about the cage with no further disturbance. Eating and drinking could be done at will. Following the operation and during the period of urine collection the birds conducted themselves in a manner that was normal in all respects.

It was soon apparent that the daily volume of urine secreted under the conditions just described was much less than might have been anticipated from the literature on the subject or from some of the data in our first two series of experiments. Of the eighteen collections the largest volume secreted in periods as long as twenty-four hours was 123.5 cc. The smallest volume eliminated in twenty-four hours was 61 cc. (table 4).

The urine that flowed from the ureters of these birds had a pH of approximately 6.22. It was a cream-colored, thick, tenacious, mucoid material containing an abundance of urcates which readily settled to the bottom of the container forming a semi-solid mass. Thick deposits of urates covered the surface of the funnel used in collecting the urine but the flow into the collecting bag was never stopped because of these deposits. We are in agreement with Das (9) that the urine of birds is usually secreted in a concentrated state.

The daily intake of water was determined for a series of normal chickens by supplying water in fountains designed to hold to a minimum loss by evaporation and waste by the bird. The volume of water consumed in

twenty-four hours by normal chickens under the conditions of our experiments was from 50 to 250 cc. (table 5). Similar observations were made on birds with exteriorized ureteral orifices. Their water consumption was not greater than that of the normal birds.

COMMENT. As has been stated evidence has been presented in the literature which indicates that birds normally secrete daily a large volume of urine. Some workers have reported for the chicken as much as 1000 cc. in twenty-four hours. If this were true either the chicken would be required to consume daily a quantity of water as great as or greater than

TABLE 4

Volume of urine collected from exteriorized ureteral orifices of chickens for periods as long as twenty-four hours

NUMBER	SEX	WEIGHT	FIRST PERIOD OF COLLECTION		SECOND PERIOD OF COLLECTION		VOLUME OF URINE, CC.		
			Duration	Volume of urine	Duration	Volume of urine	Third* period of collection (12 hours)	Fourth* period of collection (12 hours)	Total third and fourth periods (24 hours)
		kgm.		*cc.*		*cc.*			
11	M	2.20	19 hours, 5 minutes	93.5	23 hours, 45 minutes	71.0			
13	M	2.04	24 hours	123.5	16 hours, 45 minutes	76.0			
14	M	2.2	23 hours, 55 minutes	115.0	24 hours	102.0			
15	M	1.95	19 hours, 23 minutes	52.0	24 hours	87.5	36.0	30.0	66.0
16	F	2.24	16 hours, 10 minutes	73.5	24 hours	63.5	31.0	30.0	61.0
17	M	1.52	19 hours, 40 minutes	57.5	21 hours	91.5	35.5	31.0	66.5
18	M	2.24	21 hours, 10 minutes	76.0	23 hours, 45 minutes	98.0	53.5	47.0	100.5

* At the end of the third period the collecting bag was removed and another immediately applied. The total value for the third and fourth periods represents the urine output for twenty-four hours.

Average volume collected for all periods of twenty-four hours or approximately twenty-four hours was 86.77 cc.

the volume of urine secreted or it would be necessary for the mucosa of the cloaca to reabsorb a large quantity of water. Davis has stated that most of the water excreted with the urine of birds is reabsorbed in the cloaca. If such were the case selective absorption of water by the cloacal mucosa would seem to be necessary since, in the absence of such a mechanism, the dissolved urates and other salts would be returned to the blood stream and their concentration in the blood excessively augmented. This, in turn, would increase the work of the kidneys since they would be required to

deal repeatedly with a given quantity of urates. It seems reasonable to assume that the concentration of urates and other excretory products is the function of the kidneys of birds as in other animals and not the function of the avian cloacal mucosa. The data presented in this paper indicate that the normal daily volume of urine secreted by chickens is relatively small as is likewise true of the daily intake of water. These findings lend support to our belief that elimination of urine in birds is not fundamentally

TABLE 5

The daily intake of water in cubic centimeters by a series of chickens for twenty-three successive days

BIRD NUMBER	DATE AND RANGE OF TEMPERATURE IN DEGREES FAHRENHEIT							
	70-84 Sept. 1	70-85 Sept. 2	71-86 Sept. 3	70-82 Sept. 4	70-84 Sept. 5	68-84 Sept. 6	68-82 Sept. 7	72-85 Sept. 8
9	190	200	215	190	175	200	200	175
10	190	200	200	200	200	175	175	175
11*	225	225	200	200	200	250	200	200
13*	175	200	175	175	180	200	225	200
15*	150	200	150	150	200	200	150	150
	68-83 Sept. 9	70-84 Sept. 10	72-84 Sept. 11	72-84 Sept. 12	70-84 Sept. 13	68-84 Sept. 14	68-82 Sept. 15	70-82 Sept. 16
9	175	175	190	175	175	175	175	175
10	175	175	175	175	250	150	175	180
11*	180	190	185	180	225	160	180	175
13*	150	175	200	175	200	175	175	175
15*	175	175	175	175	200	180	180	180
	68-82 Sept. 17	64-70 Sept. 18	65-70 Sept. 19	64-72 Sept. 20	68-82 Sept. 21	68-80 Sept. 22	68-82 Sept. 23	
9	175	150	65	75	100	50	100	
10	155	160	65	70	125	135	150	
11*	175	165	75	65	100	70	130	
13*	175	175	50	70	115	75	90	
15*	175	150	75	75	100	100	125	

* Birds with exteriorized ureteral orifices.

different from what it is in the mammal, that whereas the urine is collected by the bladder of mammals the cloaca performs that function in birds.

Normally the urine of the chicken is in a concentrated state as it issues from the ureters but the secretory process appears at times to be easily affected by a relatively mild disturbance of the bird. Bringing chickens to the laboratory and preparing them for observation appeared to be sufficient in the case of a number of chickens to cause considerable diuresis. This observation would indicate that the normal daily output of urine in chickens cannot be satisfactorily determined by calculations based on the output during short periods of collection.

SUMMARY AND CONCLUSIONS

Observations on the urine secretion of chickens have been made by the use of three methods, two of which have been used previously: 1. The ureters were cannulated under local infiltration anesthesia and the output of urine was determined for limited periods. 2. Similar observations were made by the catheter (funnel) technic of Davis and of Coulson and Hughes. 3. The ureteral orifices were exteriorized by operative procedures described in this paper and the urine output was measured for periods as long as twenty-four hours.

The water intake of normal birds and of birds with exteriorized ureteral orifices was determined. The normal daily intake of water and likewise the normal daily volume of urine secreted by the chicken was small. In eighteen collections from seven birds the largest volume secreted in periods as long as twenty-four hours was 123.5 cc. The daily intake of water ranged from 50 to 250 cc. The normal urine of birds is a cream-colored, thick, tenacious, mucoid material containing an abundance of urates which readily settle out forming a semi-solid mass. The pH of samples taken under different experimental conditions varied from 6.22 to 6.7.

The degree of disturbance incident to preparing the chicken for observations on urine secretion was apparently sufficient to cause considerable diuresis in some birds.

Statements in the literature to the effect that the water in the urine of birds is absorbed by the mucosa of the cloaca are open to question. The evidence presented in this paper would indicate that the cloaca of the bird is analogous to the bladder of the mammal and under normal conditions the urine is concentrated by the kidneys and stored in the cloaca in whatever state it is received from the ureters. The concentration of urine in the cloaca would presuppose selective absorption of water by the cloacal mucosa which judging from our data does not appear to be a necessary hypothesis.

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ABSORPTION OF CHLORIDE AGAINST CONCENTRATION GRADIENTS FROM SMALL INTESTINES OF RATS AND OF RABBITS[1]

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Following the earlier work of Katzenellenbogen (1) and Goldschmidt and Dayton (2), during the past few years, Visscher and his co-workers (2–9) have published a series of studies on the mechanism by which living systems perform osmotic work, i.e., move substances against concentration gradients. The particular process with which they have concerned themselves has been the absorption into the blood of univalent ion salts from the dog small intestine. As a result of considerable investigation, they have reached the conclusion that, "In general, a relatively poorly absorbed substance, such as a polyvalent ion salt or an organic molecule of large molecular weight, must be present along with a univalent ion salt of ready permeability in order that the latter substance shall be transported against diffusion forces." Under such circumstances absorption can occur against a 200:1 concentration ratio. Since all the data thus far published have been obtained with dogs as the subjects, it was considered of value to determine whether or not such active absorption occurs under similar circumstances from the small intestines of other common experimental animals. In the present investigation, accordingly, we have studied the absorption of chloride against a concentration gradient from a solution containing sodium chloride and sodium sulfate placed in the small intestines of white albino rats and of rabbits.

PROCEDURE. *A. Rats.* The animals were usually starved 18 to 24 hours before being subjected to operation. Under nembutal anesthesia (40–50 mgm. per kgm. of body weight intraperitoneally) they were first tracheotomized, after which the peritoneal cavity was opened by a midline incision. Small glass cannulas were inserted into each end of a loop consisting of about 40 cm. of the aboral end of the small intestine. Ringer's solution warmed to 37–39.5°C. was washed through the loop from upper to lower end until the washings returned clear, whereupon the cannulas

[1] Assistance in the preparation of these materials was furnished by the personnel of Work Projects Administration, Official Project no. 665-71-3-69, Sub-Project no. 235.

were removed and the abdominal wall was closed with a clamp. After a wait of 30 minutes, during which the animal was kept warm, 5 cc. of test solution, likewise at a temperature of 37–39.5°C., were injected into the loop with syringe and needle in the following manner: The needle was inserted carefully into the lower end of the loop and secured with a ligature tied once. This knot was kept tight until the needle was withdrawn at the conclusion of the injection, whereupon it was converted into a double knot which then formed the lower end of the loop. The abdominal wall was again closed, and after any selected absorption period the contents of the loop were removed by syringe and needle aided by gentle milking of the loop. The volume of the contents was noted, their chloride concentration determined, and the length of loop measured. During all manipulations efforts were made to minimize trauma to the intestines or interference with their blood supply.

The tracheotomy was found necessary to prevent death or respiratory embarrassment due to obstruction in the laryngeal region. Absorption as followed in these experiments was not influenced by tracheotomy.

B. Rabbits. The procedure in the case of the rabbits differed in a few particulars from that described above for the rats. 1. Urethane anesthesia (5.5 to 6.5 cc. of 20 per cent solution per kgm.) was found to be more satisfactory than nembutal and no tracheotomy was necessary. 2. No preliminary washing with Ringer's solution was carried out because it was discovered in several experiments that enough Ringer's solution remained even after a wait of 45 minutes to produce great inequality in chloride concentration within the loop after the test solution had been added. Instead, therefore, the washing was carried out with the test solution itself, at the conclusion of which process the loop was allowed to be filled (but not distended) with the solution. By measuring the volume of the washings one could determine the minimum volume of test solution in the loop. 3. Absorption was followed by analysis of 1 to 2 cc. samples taken at appropriate intervals from the loop by syringe and needle.

Chlorides were determined by the method of Van Slyke (10). A few plasma chloride analyses were performed to verify the approximate accepted range of values.

RESULTS. *A. Rats.* Figure 1 contains a graphical representation of the results for 55 experiments in 44 of which the test solution consisted of approximately one-half isotonic sodium chloride (78.5 millimols per liter) and one-half isotonic sodium sulfate, and in 11 of which it consisted of one-half isotonic sodium chloride without any sulfate; 49 of the 55 rats were males with an average weight of 270 grams. The 6 females included in the group displayed the same behavior with respect to chloride absorption as did the males. Table 1 contains data for four typical experiments.

It is seen from figure 1 that under the circumstances of these experiments

chloride was absorbed against an increasing concentration gradient when in the presence of sulfate ion, the chloride ion concentration in the gut decreasing with time. However, when sulfate ion was absent, the chloride ion concentration in the gut solution rose to a level somewhat above that of the plasma. It will also be noted that at zero time the chloride concentration of the fluid in the intestines was, with one exception, greater than in the test solution itself (78.5 mM/L.), and that the volume at zero time was also greater than the volume of test solution injected (see table 1 for typical example). These two facts indicate that, strictly speaking, the solution in the gut at zero time was actually a variable mixture of 5 cc. of test solution plus the unabsorbed portion of the wash solution (Ringer's) plus whatever substances had entered the loop between the washing of the loop and the injection of test solution into it. This variability probably increases rather than decreases the importance of the fact

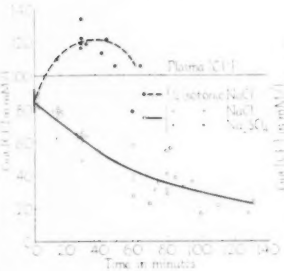

Fig. 1

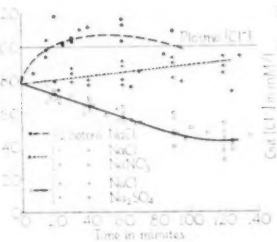

Fig. 2

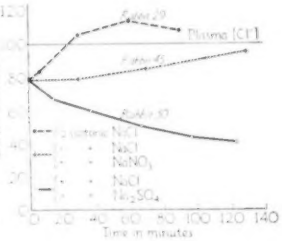

Fig. 3

Fig. 1. Absorption of chloride from small intestine of rats.
Fig. 2. Absorption of chloride from small intestine of rabbits.
Fig. 3. Three typical rabbit experiments.

that for the immediately subsequent course of the chloride concentration, the presence or absence of sulfate ion is decisive under the conditions of these experiments.

B. Rabbits. Experiments were performed on 47 rabbits; but due to variations in technique, results are presented for only 20 consecutive experiments in which the procedure was uniform. In 10 of these the test solution consisted of approximately one-half isotonic sodium chloride and one-half isotonic sodium sulfate, in 5 it consisted of approximately one-half isotonic sodium chloride only, and in 5 it consisted of approximately one-half isotonic sodium chloride and one-half isotonic sodium nitrate. The animals ranged in weight from 1.6 kgm. to 3.9 kgm., but 15 of the 20 weighed less than 2.3 kgm. The results are plotted in figure 2, experimental points on the graph representing all chloride determinations carried out for these animals. As in the case of the rats, chloride ion was absorbed

against an increasing concentration gradient in the presence of sulfate, the chloride ion concentration falling with time; but in the absence of the latter ion, the chloride concentration of the gut fluid rose to a level above that of the blood. When the bivalent sulfate ion was replaced by the univalent nitrate ion, the chloride concentration also increased but not as rapidly as when chloride alone was present.

Figure 3 contains three typical experiments, one for each test solution. The secondary decrease in chloride concentration in the case of the animal in which the test solution consisted of one-half isotonic sodium chloride only was the rule. In some animals this drop continued until the chloride concentration fell to a value below that of the plasma chloride. The explanation of this phenomenon is not apparent, but it is probable that conditions for chloride impoverishment similar to those under investigation came to prevail spontaneously in these preparations. In figure 1, there

TABLE 1

Four typical rat experiments

RAT	WEIGHT	SEX	TEST SOLUTION	$[Cl^-]$	VOLUME INJECTED	VOLUME RECOVERED	$[Cl^-]$	TIME	LENGTH OF LOOP
	grams			*mM/L.*	*cc.*	*cc.*	*mM/L.*	*min.*	*cm.*
49	225	♂	½ NaCl ½ Na_2SO_4	78.5	5	5.6	84.9	0	42
64	270	♂	½ NaCl ½ Na_2SO_4	78.5	5	4.0	60.4	30	25
42	220	♂	½ NaCl ½ Na_2SO_4	78.5	5	2.5	38.4	60	31
111	270	♂	½ NaCl only	78.5	5	2.3	117.4	30	40

is some suggestion that a similar situation obtains in rats as well as in rabbits.

Discussion. These results have been derived from so-called "acute intestinal loop" experiments by a technique which involves deviations from normal physiological conditions. In particular the effects of anesthesia, of manipulation of the intestines, and of interference with blood supply must be taken into account. In this connection it may be mentioned that Dennis (11) has shown chloride impoverishment from solutions containing sulfate ion placed in Thiry-Vella loops of unanesthetized dogs, and that Clark (12), in work to be published, has demonstrated rapid chloride impoverishment in absorption experiments by a rinse method on a few intact rats. The qualitative nature of the results of the present acute loop experiments is, therefore, not to be ascribed to the particular technique employed.

Volume changes have not been emphasized in reporting the results

inasmuch as such alterations are the resultant of both absorption and secretion, and inasmuch as there is no reliable method of estimating the latter. Our main interest in volume has been to ascertain that when chloride concentration decreased, it was not a dilution effect, but rather that an actual absorption of chloride had occurred as evidenced by volume reductions. Actually such volume decreases invariably did take place.

As a final point, it may be mentioned that the highest blood-gut chloride concentration ratios attained in these experiments have been almost 10:1 for rats and about 3:1 for rabbits, as contrasted with a 200:1 ratio reached with dogs under rather comparable circumstances. Apparently the dog is a more suitable experimental animal for studying this phenomenon under these conditions if rapid chloride impoverishment is desired.

SUMMARY

1. Chloride is absorbed against a concentration gradient from a solution containing approximately one-half isotonic sodium chloride and one-half isotonic sodium sulfate placed in the small intestines of white albino rats and of rabbits.

2. Blood-gut chloride concentration ratios reached are much lower than those reported for dogs under comparable conditions.

Note. I should like to acknowledge my indebtedness to Dr. M. B. Visscher for suggesting this study and to Mr. Courtland Robinson for his technical assistance in the performance of many of the experiments.

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STUDIES ON RAT BRADYCARDIA

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In 1929 Carter and Drury reported that pigeons fed exclusively on polished rice developed a bradycardia and partial heart block, vagal in origin, prior to the onset of polyneuritis. Subsequently Carter (1930) claimed that the cause was a deficiency of some factor other than thiamin. Drury, Harris and Maudsley (1930) found that rats maintained on a thiamin-deficient diet developed bradycardia. From paired-feeding experiments they concluded that inanition was not a factor, although they did not feed a group *ad libitum* concurrently with the paired groups. They stated that the heart rates of starved rats returned to normal if thiamin was supplied. However, Parade (1938a, b) reported that rats on low food intakes developed bradycardia even if given adequate supplies of thiamin and contended that the vitamin cured deficiency bradycardia by increasing appetite. Zoll and Weiss (1936) found that changes in the ST segment and T wave, present in rats showing bradycardia, were abolished by giving thiamin to fasting animals.

The cure of bradycardia in deficient animals has been employed as a means of assaying the thiamin potency of foods and biological material. Claims have been made for the specificity of the method but the effects of changes in the composition of the diet have not been studied. It has been our purpose to investigate the relation of other B vitamins, of choline, of food consumption, and of fat intake upon the development of bradycardia in rats.

METHODS. The animals used in the experiments were young albino rats of the Wistar strain, weighing from 40 to 60 grams, from the Connaught Laboratories Farm colony. During the experimental period the rats were housed in individual cages with wire-mesh bottoms and a supply of water was always available. The heart rate was obtained from an eight-second tracing taken by a Cossor-Robertson cathode ray electrocardiograph. Electrodes used were 24-gauge subcutaneous needles inserted to a depth of 2 to 3 mm. Rats were held in a prone position by four nooses, or by four lumps of plasticine, one to each paw. Two minutes were allowed for the rat to become quiescent and lead II was ordinarily used. The average heart rate of young rats was found to be 570.

We were unable to carry our groups below an average heart rate of 400 without considerable mortality and a rate of 420 to 430 was considered to be satisfactory bradycardia.

Diets were planned to be free of constituents of the vitamin B complex and of choline. The compositions of the diets were as follows:

DIET	DIET 1	DIET 2	DIET 3	DIET 4
	per cent	*per cent*	*per cent*	*per cent*
Casein (Labco—vitamin free)	20	10	20	20
Corn oil (Mazola)	10	0	0	20
Sucrose	64	84	74	54
Salts (Steenbock and Nelson no. 40)	4	4	4	4
Agar	2	2	2	2
Cod-liver oil concentrate	0.015	0.015	0.015	0.015

EXPERIMENTAL RESULTS. *Effect of various vitamin B complex supplements on rat bradycardia.* In the following two experiments the plan of procedure was as follows. A group of rats (60 in the first experiment and 50 in the second) was placed on diet 3 and the heart rates taken at intervals. When a sufficient degree of bradycardia had developed, the rats were arranged in groups with equal average heart rates, after rejection of those whose rates were too high or too low. Various supplements were then injected and the heart rate was obtained 24 hours after injection. Amounts of supplements used were thiamin 25 γ, choline 20 mgm., flavine 20 γ, nicotinic acid 0.1 mgm., vitamin B_6 (crude solution) 1.0 cc., and filtrate factor (crude solution) 1.0 cc.

From the table it may be observed that choline or flavine, alone or in combination, nicotinic acid, vitamin B_6 or the filtrate factor did not diminish the bradycardia and that choline or flavine alone or in combination did not increase the effect of thiamin. In the last series the rates in the thiamin-no food group were 460 and 450 three and five hours after injection. The transitory rise in the thiamin-no food group was not as high as in the thiamin-food group and it was not maintained. Presumably the partial response was due to food present in the gastro-intestinal tract of the rat at the time of injection.

The effect on the heart rate of daily injections of 12.5 γ of thiamin, 10.0 γ of flavine, and 10.0 mgm. of choline, either separately or in various combinations, was studied. Groups of 10 rats fed diets 1 and 2 were used. They were paired as to food intake with control groups receiving no supplements to eliminate the variable factor of food consumption. It was found that thiamin was the only supplement of those tested that prevented bradycardia and that choline or flavine, alone or in combination, did not prevent the bradycardia nor increase the effect of thiamin when given with it.

Effect of food intake on rat bradycardia. Twenty-one rats were divided into 3 groups of equal weight and sex. The first group received diet 3 *ad lib.*, the second was paired with the first as to food intake and received daily injections of 12.5γ of thiamin, and the third group was given diet 3

TABLE 1

Injection of vitamin B supplements to vitamin B deficient rats

	BEFORE	24 HOURS AFTER
First series		
Controls (saline)	433 ±8 (7)	419 ±5 (7)
Thiamin (no food)	431 ±7 (7)	346 ±16 (6)
Thiamin + food	433 ±8 (7)	544 ±5 (7)
Choline	433 ±8 (7)	373 ±16 (7)
Flavine	431 ±10 (7)	348 ±12 (7)
Choline + thiamin	431 ±10 (7)	532 ±6 (7)
Thiamin + flavine	431 ±9 (7)	515 ±7 (7)
Thiamin + flavine + choline	431 ±8 (7)	504 ±5 (7)
Second series		
Controls (saline)	399 ±7 (7)	385 ±9 (7)
Thiamin (no food)	399 ±9 (7)	214 ±23 (7)
Thiamin + food	390 ±15 (7)	510 ±6 (7)
Nicotinic acid	405 ±6 (5)	370 ±15 (5)
Vitamin B_6	412 ±14 (5)	382 ±16 (5)
Filtrate factor	426 ±15 (5)	410 ±4 (5)

The probable errors of the means were obtained by the following formula. P. E. $= 0.6745 \sqrt{\frac{\Sigma d^2}{n(n-1)}}$. The figures in parentheses give the number of animals used for the observation.

TABLE 2

Effect of daily injection of thiamin to paired-fed rats and of 5 per cent yeast diet given ad lib. on the heart rate

	1ST	9TH	15TH
Deficient group	569 ±7 (7)	528 ±7 (7)	431 ±12 (7)
Paired + thiamin	578 ±5 (7)	539 ±5 (7)	510 ±11 (7)
Ad lib. 5 per cent yeast diet	570 ±8 (7)	544 ±10 (7)	600 ±5 (7)

ad lib. to which had been added 5 per cent by weight of dried brewer's yeast. Heart rates were taken on the 1st, 9th, and 15th days (table 2).

As the heart rates were significantly higher in the *ad lib.* group, the relation of food intake was investigated in the following experiment. Fifty rats were divided into 5 groups of equal weight and sex and fed as follows. The first group received diet 3 *ad lib.*; the second was paired with the first as to food intake and received 12.5 γ of thiamin by injection; the third

was paired with the first, using the basal diet plus 5 per cent yeast; the fourth was given diet 3 *ad lib.* plus 12.5 γ of thiamin by injection daily; and the fifth *ad lib.* supplies of the same diet as group 3 including yeast. Heart rates were taken on the 7th, 16th and 21st days. The results are given in figure 1. The quantity of yeast given to the rats furnished approximately the same amount of thiamin as was supplied as a supplement to the other group.

The graph indicates that, in the above amounts, thiamin was as effective as whole yeast in maintaining the heart rate in the paired-fed groups, while in the *ad lib.* fed groups it was almost equal in effect. The food intake was greater in the *ad lib.* 5 per cent yeast group than in the *ad lib.*

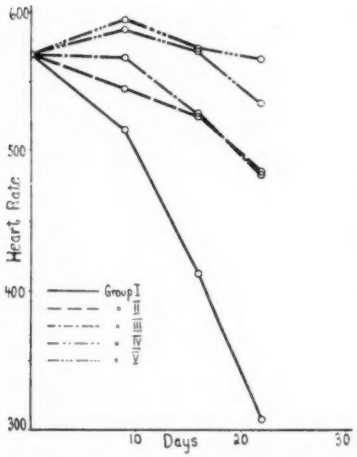

Fig. 1. Comparative effects of paired and *ad lib.* feeding on heart rate in rats.

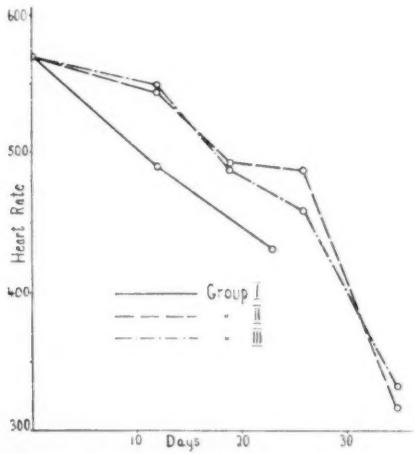

Fig. 2. Comparative effect of diets with and without fat on heart rate in rats.

basal diet plus thiamin group. The lower rates in the groups on paired feeding suggest that a restriction in food intake is also a factor in the production of rat bradycardia.

Effect of dietary fat on rat bradycardia. Since the inclusion of fat in a diet deficient in thiamin has been shown to delay the onset of polyneuritis, weight loss and other manifestations of thiamin deficiency (Evans and Lepkovsky, 1928), the effect of fat in the diet was next investigated. As choline has been shown to be necessary for the maintenance of a normal level of liver fat (Best and Huntsman, 1935) and flavine to be related to fat deposition in the depots (McHenry and Gavin, 1938), the effects of these two supplements were also studied.

Thirty rats were divided into 3 groups of equal weight and sex. They

were fed *ad lib.* and the amount of food consumed was weighed. The first group was fed diet 3 and was given 10.0 mgm. of choline and 10.0 γ of flavine by injection daily; the second group received diet 4 and no supplements; and the third group received diet 4 and the supplements given to the first group. Heart rates were taken on the 12th, 19th, 23rd, 26th and 35th days of the experiment and the results are shown in figure 2.

As it appeared that fat had an effect in delaying the onset of bradycardia, various percentages of dietary fat were investigated. Yeast autoclaved for 6 hours at pH 9 at 15 pounds' pressure was given as supplement to supply those B vitamins which do not influence heart rate but which are concerned with fat transport and storage (McHenry and Gavin, 1938).

Fifty rats were paired by weight and sex into 5 groups. These were fed *ad lib.* the following diets.

Diets

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Casein	20	20	20	20	20
Hydrogenated cotton-seed oil (Crisco)	0	10	20	30	40
Sucrose	70	60	50	40	30
Salts	4	4	4	4	4
Agar	2	2	2	2	2
Autoclaved yeast	4	4	4	4	4
Cod-liver oil concentrate	0.015	0.015	0.015	0.015	0.015

Electrocardiograms were taken on the 1st, 13th, 21st, 27th, 35th, 42nd and 48th day and the results are shown in figure 3. On the 27th day the no-fat group was placed on the 40 per cent fat diet. The numbers in brackets indicate the number of surviving rats when the rate was obtained.

On the 27th day, the last day on which we can compare all groups, the least drop in heart rate had occurred in those on the 20 per cent diet. From the survival value of the diet and maintenance of heart rate until the experiment was terminated, the 30 per cent fat diet was the most effective. After the administration of fat to the group previously receiving none, the rapid development of bradycardia was checked and the heart rate one week later was about 100 more than would be indicated by extrapolation. It is seen that as the fat content of the diet is increased up to 30 per cent the onset of bradycardia is delayed.

Studies were made of the electrocardiographs of the rats in the above groups, the PR and QRS interval being measured and the ST segment and T wave examined. No regular changes of significance were noted until the rate was well below 400 in each group at which time many of the rats

of the group had died. Of the 6 surviving members of the 30 per cent fat group on the 48th day 4 had rates below 400 and of this number 3 showed depression of the ST segment and 1 a raised ST segment while the T wave was inverted in one and negative in another. Of the 4 surviving members of the 40 per cent fat group on the 48th day 3 had rates below 400, one one had a depressed, one a raised, and the other an isoelectric ST segment. In the no-fat group which had an average rate of 385 on the 27th day with 9 survivors there were no ST or T changes. It would appear that changes in the electrocardiograph complex occurred in the premortal phase, and were not uniform.

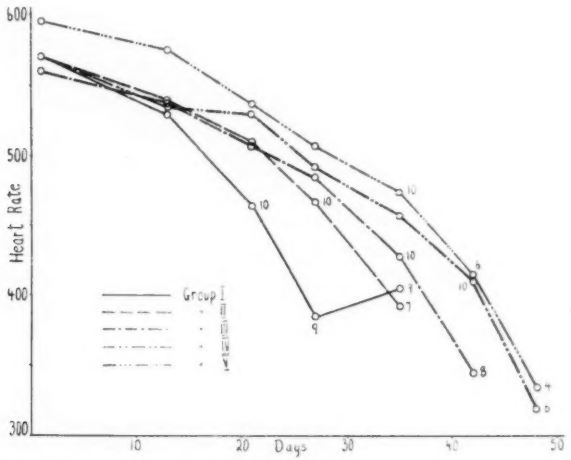

Fig. 3. Effects of various proportions of dietary fat on heart rate in rats

Discussion. Since none of the other vitamin B supplements influenced the heart rate, the effect of thiamin upon bradycardia appears to be specific. However, the maintenance of normal heart rate by thiamin depends upon food consumption. Our results, in this respect, are in agreement with those of Parade (1938a). A reduction in the amount of thiamin or of food slows the heart rate. The administration of the vitamin to deficient rats does not restore the heart rate unless food is also supplied.

This correlation between thiamin intake, caloric intake and heart rate indicates that bradycardia is the result of an inadequate supply of available nutrient for the sinus node rather than a toxic effect of accumulated metabolites. This conception agrees with the findings of Lu (1939), Drury *et al.* (1929, 1930), and Kalaja *et al.* (1938).

The classical work of Evans and Lepkovsky on the thiamin-sparing action of fat has been confirmed by many workers with regard to delay in

the onset of polyneuritis and for survival value of a diet but the relation of dietary fat to bradycardia has not been previously investigated. Our observations indicate that dietary fat spares the need for thiamin to prevent bradycardia. This would be of importance in the assay of the thiamin potency of foods by the bradycardia technique, as a food containing a large percentage of fat would influence the heart rate on that account.

SUMMARY

The relationship of various B vitamins, food intake and dietary fat to rat bradycardia has been investigated.

From the effect of injections of various B vitamins to deficient rats; from the effect of daily injections of various B vitamins to paired-fed rats; and from the equal effects of thiamin and yeast on paired and *ad lib.* diets it is apparent that thiamin alone of the members of the vitamin B complex investigated is effective in reducing rat bradycardia.

That bradycardia is not solely the result of inanition was demonstrated in paired feeding experiments; animals receiving thiamin had a rate considerably above those of the basal group. However, the quantity of food consumed was shown to be a factor in causing bradycardia by contrasting groups fed *ad lib.* with those restricted in food supply. Bradycardia is not relieved by administration of thiamin unless food is also given.

A sparing action of dietary fat upon thiamin requirement for the prevention of bradycardia has been shown.

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ACTIVITY PATTERNS OF THE RESPIRATORY NEURONS AND MUSCLES[1]

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Systematic electrical sounding of the medulla and cord have revealed the participation of many neural units in the respiratory act—sensory, motor and intermediate nerve cells (Gesell, Bricker and Magee, 1936). Our present experiments inquire into the nature of the activity of these units. Since the adjustment of respiratory contractions represents the end result of the nervous integration of the respiratory act, its explanation becomes our ultimate objective.

PROCEDURE. Fortunately, muscular activity is readily followed with closely approximated floating electrodes resting on the upper surface of exposed muscles. When selectively applied for detecting discharges of single muscle units they yield signals which are readily analyzed for number and frequency. Less carefully applied they yield potentials in denser fusillades which are recorded as dark shadows of characteristic shapes, indicative of the forces which are at work (Gesell, 1936b). The related central potentials are registered with the bipolar sounding technique mentioned above (Gesell, Bricker and Magee, 1936). Dogs were used exclusively.

As a matter of economy all potentials were recorded on bromide paper. This procedure had the advantage of permitting a large number of observations but the disadvantage of giving closely packed potentials, for the insensitive emulsion requires a relatively slow movement of the roll. To obtain accurate measurements for the establishment of the frequency curves it was, therefore, desirable to make ten-fold photographic enlargements of the original records. The reconstructed records were made in this way. To conserve illustrative space the inspiratory periods are indicated in the reconstructed records by a thickening of the base line of the electrogram. (Compare the original and reconstructed records in figs. 1 and 2.)

RESULTS. So far as we know these experiments represent the first attempt to follow a physiologically organized train of signals through a

[1] This study was supported by a grant from the Rockefeller Foundation.

complicated chain of neurons. Proposing, as we do, an inherent rhythmical activity of the respiratory mechanism (Gesell, 1939) and bearing in mind the production of proprioceptive potentials by respiratory contractions, it seems more logical to begin our observations in the motor end organs rather than in the peripheral sensory end organs. Records will, therefore, be presented from one station to another in the antidromic order.

A preliminary consideration of the physiological source of potentials found at the various anatomical stations will assist in the interpretation of our records. For example, potentials occurring in phase with inspiration may be logically expected to arise from: 1, motor discharges in the reticular system and motor nuclei activating inspiratory muscles; 2, stretching of Golgi endings in contracting inspiratory muscles; 3, stretching of Golgi endings in passively stretched expiratory muscles; 4, passive stretching of expiratory muscle spindles; 5, stretching of vagal pulmonary endings, and 6, sensory stimulation of the upper respiratory tract by tidal air.

On the other hand potentials occurring in phase with expiration may be expected to arise from: 1, motor discharges in the reticular system and motor nuclei activating expiratory muscles; 2, stretching of Golgi endings in contracting expiratory muscles; 3, stretching of Golgi endings in passively stretched inspiratory muscles; 4, passive stretching of the inspiratory muscle spindles; 5, sensory stimulation of the upper respiratory tract by tidal air.

Since the periodic sensory potentials depend upon the specific mechanical conditions set up by respiratory contractions, precise information of the nature of these contractions is essential to our problem. It is obvious that the firing of the Golgi endings in the muscle tendons should increase with rising muscle tension just as the firing of the vagal endings augments with expanding volume of the lungs. Accordingly identical patterns of activity in the motor and sensory portions of the respiratory reflex arc would not be unexpected.

Our first record is that of diaphragmatic contraction with the electrodes well separated to combine all regional potentials in dense fusillade form (see fig. 1). The picture is that of a serrated triangle, the characteristic form of inspiratory contraction already reported by Gesell (1936). The steadily rising shadow depends in the main upon an increasing summation of coinciding potentials of contiguously contracting elements. Such coincidence must of necessity increase with increasing frequency of muscle fiber twitch and with increasing recruitment of the muscle elements, known to occur with increasing strength of muscular contraction (Adrian and Bronk, 1928; Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932; Bronk and Ferguson, 1935; Gesell and White, 1938). More rapidly decreasing frequency and decruitment produce the rapidly falling shadow of expiration. The gradients spell the rate and extent of waxing and wan-

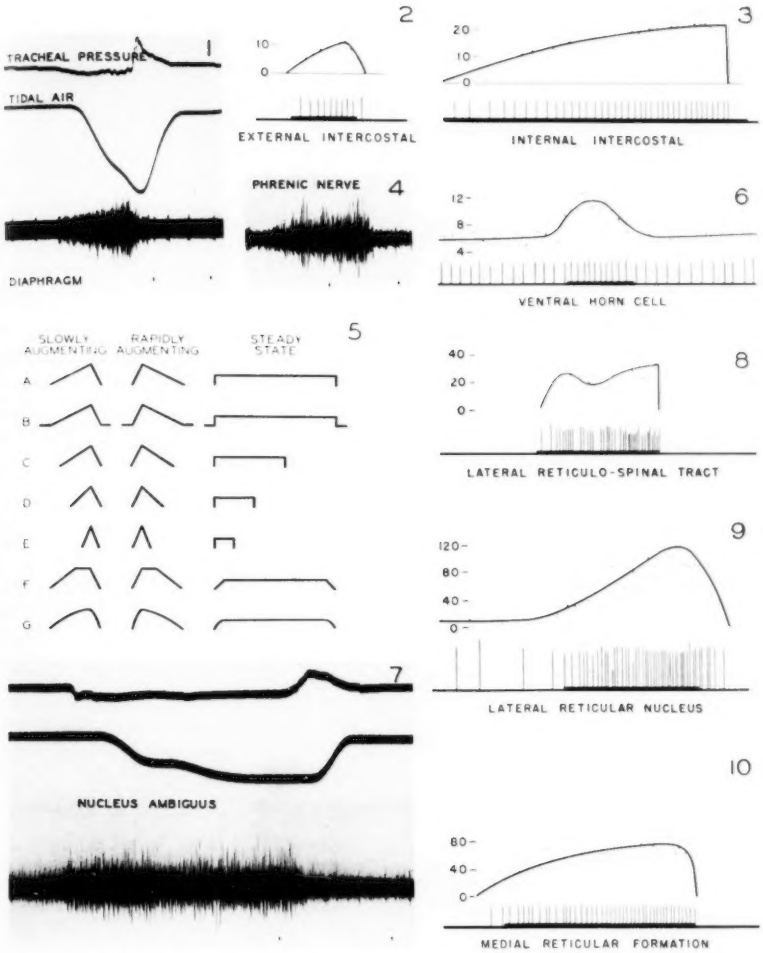


Fig. 1. An original record of a fusillade of inspiratory potentials recorded from the diaphragm of the dog. In the tracings of tracheal pressure and tidal air, downstrokes represent inspiration, upstrokes expiration. The time interval of one second is indicated by the two short vertical marks in this and all subsequent records in which frequency of discharge is not plotted.

Figs. 2 and 3. Electromyograms of inspiratory contractions of the external and internal intercostal muscles and their accompanying curves of frequency. The thickening of the base line of the electromyogram indicates the period of inspiration.

Fig. 4. Original fusillade record of the phrenic nerve.

Fig. 5. A schema showing the variations in the three primary types of activity patterns found throughout the respiratory arcs. For further description see page 618.

Fig. 6. Electrical discharge and its frequency curve of a ventral horn cell during the phase of inspiration.

Fig. 7. Original fusillade record of the discharge of the nucleus ambiguus during the phase of inspiration.

Fig. 8. Electrical discharge and its frequency curve of the lateral reticulo-spinal tract.

Figs. 9 and 10. Electrical discharges and their frequency curves of the lateral reticular nucleus and medial reticular formation.

ing power. The slowly rising gradient corresponds with the slow filling of the lungs represented in the gentle decline of the tidal air and tracheal pressure records and the rapidly falling gradient conforms with the sudden emptying of the lungs as shown in the steep incline of the tidal air and tracheal pressure records.

Our second figure shows unicellular activity in the external intercostal muscle. It will be seen that this particular unit opens fire early in the phase of inspiration and closes in the early stage of expiration. Frequency waxes and wanes in the manner shown in the graph. The triangular form of the curve forces us to conclude that both acceleration and recruitment possess a common pattern approaching a triangular outline. An exceptionally striking example of this pattern of frequency discharge is illustrated in figure 3, from the internal intercostal muscle. The curve rises slowly along an approximately straight gradient from 0 to 20 per second and then drops abruptly along a perpendicular to zero at the end of inspiration. Though the contraction of this fiber group appears late in the phase of inspiration, it shows the same acceleration of frequency as those fibers beginning earlier. It is of further interest to note that this record is taken from the internal intercostal muscle thus making it the third variety of inspiratory muscle exhibiting the triangular configuration of activity. From figures 1, 2 and 3 and innumerable observations on other inspiratory muscles we conclude that inspiratory muscles have a common basic pattern of activity, a slowly increasing activity during the period of inspiration and a more rapidly decreasing activity during the period of expiration.

Rising gradients of the myoelectrograms are safely interpreted as indication of increasing muscle activity and falling gradients the reverse. We believe that similar changes in the electrical discharges of the nerve cells of the reflex arc have the same significance. So in the fusillade obtained from the phrenic nerve (see fig. 4) we see a slowly increasing activity of the ventral horn cells during inspiration followed by a speedy return to rest during expiration. We may, therefore, anticipate a most significant conclusion that inspiratory activity throughout the motor and sensory stations of the respiratory arc is similar to that in muscle, subject to minor variations as schematically illustrated in figure 5. If, for example, the discharge is a continuing one, which fails to cease during the expiratory pause, it assumes the general appearance of pattern B. Or if breathing is accelerated, the patterns change progressively to C, D, and E. In the final stage, due to the shortening of the inspiratory phase, the configuration approaches an isosceles triangle. If increasing power of contraction is suddenly arrested it results in a distinctly trapezoidal type (see pattern F). If the trapezoidal pattern is rounded as often happens in ordinary breathing, pattern G is the result. Any of the patterns A, C, D, E, F, and G may

occur upon a continuing base as schematized in pattern B. For reasons which we believe will be apparent as the results are presented, these varying patterns come under one general heading which will be referred to in the future as the "slowly augmenting" type.

Figure 6 differs in several ways from the basic triangular type A. In the first place the firing is continuous, persisting through both phases of breathing. Secondly, the curve is distinctly more rounded and, therefore, approximates most closely type G. When a type G pattern of motor activity is found (muscle or motor cell) it is logical in turn to expect identical patterns of activity in the higher respiratory stations for a type G muscular contraction implies a type G distortion in the outlying proprioceptive endings of the lungs, muscles and tendons capable of appearing as sensory potentials in the centers. Figure 14 recorded from the internal arcuate fibers, approaches such a pattern in the sensory portion of the central respiratory mechanism.

For reasons unknown, the mechanical act of inspiration sometimes fails to progress along its usual course. Instead, it stops midway and holds, up to the moment of expiration, the air which has been inspired. As might be expected such breathing is associated with the trapezoidal pattern of potentials (type F) with its gently rising gradient, its plateau and its sharp decline corresponding in all respects to the mechanical events just noted (see fig. 7). We shall have occasion to observe the image of this motor pattern in a sensory unicellular discharge from the parafascicular gray matter in figure 17. Other modifications of the basic pattern will be shown at different stations in the center in figures to follow.

Figure 7 is the last example of potentials from the purely motor group of structures (muscle, motor nerve and ventral horn cell). The next important group of potentials in the antidromic direction come from the reticular cells and their neuraxons making up the reticulo-spinal tracts. The first example shown in figure 8 comes from the lateral reticulo-spinal tract which relays its signals directly to the motor cells. This particular discharge is not as regular as those already described. It most probably is of the slowly augmenting type upon which an initial spurt has been superimposed. Figures 9, 10, 11 and 12[2] coming from the lateral reticular nucleus and from the medial reticular formation, however, show the basic

[2] Since the proximity of the electrodes to the source of potentials was never known, we have put little stress upon the absolute values of the input potentials, but due to the rigid fixation of the animal's head and the sturdiness of the electrode manipulator, the changes in intensity of the potentials which are seen may be valid. In figure 10 they are exceptionally uniform. Yet close inspection shows a barely perceptible reduction in amplitude in the closing potentials of each discharge. In other records to follow, much larger progressive changes and much greater variations in individual potentials will be seen. We have made no effort to account for our findings on amplitude but present them for any value they may have (see fig. 12).

configuration, with a slowly rising frequency during the inspiratory period and a rapidly falling frequency during the expiratory phase. They, too, show the same variations of continuity and discontinuity, rounding, etc., already described for the respiratory muscles and their anterior horn cells. The frequency of discharge is considerably higher than the expected rate of discharge of the anterior horn cell and its corresponding muscle fibers during eupneic breathing. This lack of timing between the final motor neuron and the reticular cells and their several varieties of synapsing sensory fibers is pertinent to the elucidation of synaptic function. It could be interpreted as supporting the theory of synaptic drive (Gesell, 1939a, b) as opposed to the theory of synaptic transmission.

Among the internal arcuate fibers, we finally enter a region devoid of motor potentials and containing sensory fibers coming from the Golgi and muscle spindle endings and pulmonary proprioceptors. It is therefore of interest that figure 13 taken from this region does show the characteristically slow acceleration and the rapid deceleration seen in the strictly motor stations of the inspiratory arcs and in the intermediate reticular system. As was the case for the reticular system, frequency of discharge is again distinctly higher than the expected frequency of eupneic muscle fiber twitch and thus serves as another example of a gross lack of correspondence of rhythm from one neural station to another.

Figure 14 while coming from the same high level as figure 13 and showing the same type of trapezoidal variation differs in that the inspiratory activity is superimposed upon a continuing discharge. As in most records from sensory stations (see those which follow) the maximum frequency of firing is considerably higher than that observed in the motor end organs during eupneic breathing.

The nucleus cuneatus, relay station for the proprioceptive signals, is generally accepted as playing a definite rôle in the regulation of breathing. It is, therefore, significant to find these most important signals conforming with the basic pattern of inspiratory contractions (see fig. 15). As was anticipated, the proprioceptive signals duplicate in a convincing way the mechanical changes in tension transpiring in the inspiratory muscles and their attachments.

Figs. 11 and 12. Electrical discharges and their frequency curves of the medial reticular formation.

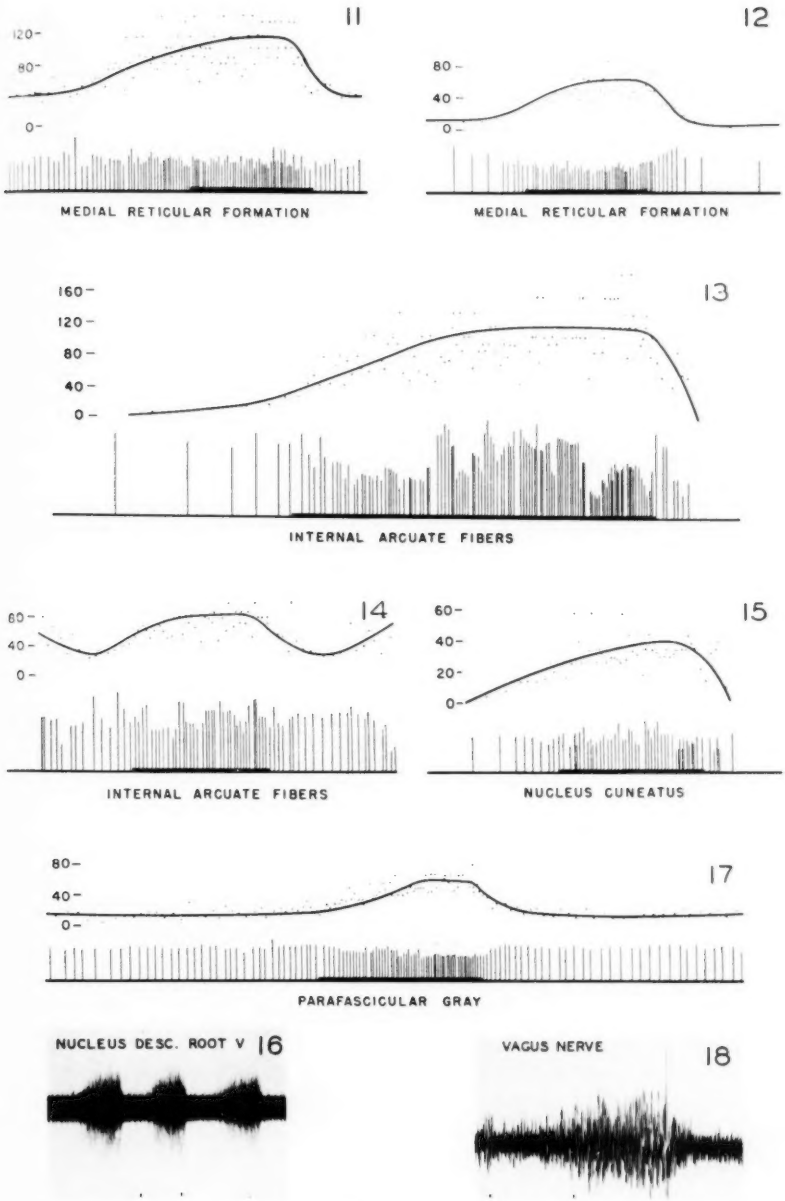
Fig. 13. Electrical discharge and its frequency curve from the internal arcuate region.

Figs. 14 and 15. Electrical discharges and their frequency curves from the internal arcuate region and nucleus cuneatus.

Fig. 16. Original fusillade record of the activity of the nucleus of the descending root of V during the phase of inspiration.

Fig. 17. Electrical discharge and its frequency curve from the parafascicular gray.

Fig. 18. Original record of the peripheral vagal discharge.


11
120
80
0
MEDIAL RETICULAR FORMATION
12
80
40
0
MEDIAL RETICULAR FORMATION
13
160
120
80
40
0
INTERNAL ARCUATE FIBERS
14
60
40
0
INTERNAL ARCUATE FIBERS
15
60
40
20
0
NUCLEUS CUNEATUS
17
80
40
0
PARAFASCICULAR GRAY
NUCLEUS DESC. ROOT V 16
VAGUS NERVE 18

Figs. 11–18

The striking fusillades seen in figure 16 come from the nucleus of the descending root of V. Those coming from the descending root had the same decisive triangular configurations. We are unable to state definitely the physiological source of these potentials.

The parafascicular gray must be a most important structure in the control of breathing for it relays the vagal stretch reflex originating in the lungs. We have eight observations on this structure, four unicellular and four fusillades. In each instance the activity of this structure increases during the inspiratory filling of the lungs and decreases during expiratory deflation. These findings are in satisfactory agreement with Adrian's results demonstrating the progressive increase of discharge of the peripheral vagal nerve with increasing lung inflation. The continuity of firing noted in the expiratory period in figure 17 is comparable to that seen in many vagal fibers during ordinary breathing. The flattening of the curve towards the close of inspiration is no doubt due to an arrested inspiration and the absence of a progressively increasing stretch of the proprioceptive endings as indicated by the original tidal air record. For further support of this mechanical explanation of the sensory events in figure 17 we may refer back to figure 7 where a trapezoidal motor discharge conforms with the accompanying trapezoidal configuration of the tidal air record.

Our last record of the inspiratory series of potentials, figure 18, brings us out into the cervical vagus nerve where the parafascicular gray potentials arise. Thus we end with the same slowly augmenting pattern of potentials in peripheral sensory nerves as that with which we began in the inspiratory muscles (figs. 1, 2 and 3).

Expiratory potentials. The activity of the expiratory muscles during eupneic breathing is definitely less than that of the inspiratory muscles. This means, first of all, that most of the energy employed for breathing is provided by the inspiratory muscles, and secondly, that relatively few expiratory muscle fibers contract during the expiratory phase (Gesell, 1936). Such being the facts, the expectancy of contacting expiratory potentials in the brain must be proportionately small as compared with the contacting of inspiratory potentials, a conclusion already reached by Gesell, Bricker and Magee (1936). Nevertheless, it has been our fortune to encounter enough expiratory discharges to follow them through the respiratory ares, as was done with the inspiratory signals. In contrast to our findings on inspiratory potentials, which show only one underlying basic pattern, the expiratory potentials show two characteristic patterns. One is the "rapidly augmenting" type in which the potentials fall off slowly. Like the inspiratory pattern it is triangular in shape but the mirror image of the slowly augmenting type (see fig. 5). The potentials build up rapidly and then fall slowly during the remainder of expiration. The other is the "steady state" type more or less rectangular in shape. The first example of the rapidly augmenting type is a fusillade discharge re-

corded from the internal abdominal oblique muscle, shown in figure 19. A unicellular discharge of the external abdominal oblique is shown in figure 20. Although figure 19 is a multicellular discharge of several fibers in which unicellular analysis is impossible, it indicates that recruitment and decruitment of the muscle fibers correspond with the rate of acceleration and deceleration of firing as plotted in figure 20.

So called rectangular contractions are seen in figure 21. They open abruptly, but once established show a marked tendency to continue at a steady state. The upper record shows both fusillade and unicellular firing of the external abdominal oblique muscle, for the electrodes happened to rest directly upon an active unit yielding powerful potentials which stand in relief below the fusillade. The lower record shows a simultaneous unicellular discharge of the external intercostal muscle. The significance of the existence of two types of expiratory contraction as opposed to one of inspiration will not be discussed in this paper.

Figure 22 is a unicellular discharge of the rapidly augmenting type from the ventral horn of the cord. It starts at the very beginning of expiration from zero, accelerates with great speed, and then decelerates more slowly back to zero just before the beginning of the oncoming inspiration. Occasionally we had the fortune of engaging both inspiratory and expiratory potentials in unicellular pattern in a single record. Such is the case in figure 23 where the potentials arose in the nucleus ambiguus. We can present no better record picturing the inherent differences of inspiratory and expiratory firing and suggesting the intimate physical relations of the oppositely functioning cells. Such proximity of antagonistic motor elements is of considerable interest in regard to the architectural arrangement of the respiratory center where inspiratory and expiratory potentials were found closely intermixed (Gesell, Bricker and Magee, 1936). The frequency of discharge of the ventral horn cells in figures 22 and 23 will be seen to conform with the expected frequency of twitch in muscle under the same eupneic conditions.

Traveling upstream towards the reticular nucleus, we found rapidly augmenting potentials in the unicellular discharge of the lateral reticulospinal tract (see fig. 24) just as we found the slowly augmenting type during inspiration. The discharge opens at zero level. It reaches a maximum of 60 per second before the lungs deflate and then subsides more slowly to zero during the expiratory pause. This maximum frequency of 60 is definitely higher than the frequency one expects to find in muscle fibers during a weak eupneic contraction and thus represents the first lack of agreement in frequency of discharge on passing from one neuron station to another in the expiratory arcs. The successive inspiratory and expiratory potentials indicate that both inspiratory and expiratory fibers descend side by side in the reticulo-spinal tracts.

The next station is the lateral reticular nucleus (see fig. 25). This

record is noteworthy for the symmetry of discharges. Rectangular in shape, they offer a striking example of central steady state firing. In this instance the frequency of firing is low enough to conform with the low rate of expiratory muscle twitch. We may then conclude from figures 24 and 25 that the expiratory reticular cells and their neuraxons may discharge at varying frequencies, some as low as and others higher than the frequency of the ventral horn cell, thus conforming with the conclusions already arrived at in the inspiratory system of nerve cells.

Another steady state pattern, but in fusillade form and from the medial reticular formation, is seen in figure 26. It will be recalled that any prolongation of the expiratory pause leads to an extension of the steady state contraction when that type of contraction prevails. This same extension is now seen in a central station. Coming from a mixed motor-sensory region, the physiological origin of the potentials must remain uncertain. They may be motor discharges of the reticular system destined for the ventral horn cells innervating expiratory muscles, or sensory signals started at Golgi endings in persistently contracting expiratory muscles, or signals initiated by Golgi endings in passively stretched inspiratory muscles, or signals from the spindles of passively stretched inspiratory muscles.

In figure 27 we come back to the internal arcuate fibers again, but this time in an expiratory arc and engage sensory potentials in the secondary sensory neuron in phase with expiration. Thus the expiratory series of potentials ends with the same rapidly augmenting type with which we began in the internal abdominal oblique (see figs. 19 and 20).

Analysis of patterns. The outstanding point of our experiments is that the three primary patterns of action potentials established for the respiratory muscles are repeated along the anatomical stations of the respiratory

Figs. 19 and 20. Electrical discharges of the internal and external abdominal oblique during the phase of expiration. The electrogram of the internal abdominal oblique has its origin from more than one group of fibers.

Fig. 21. Original electromyograms of two expiratory muscles showing fusillade and discrete discharges.

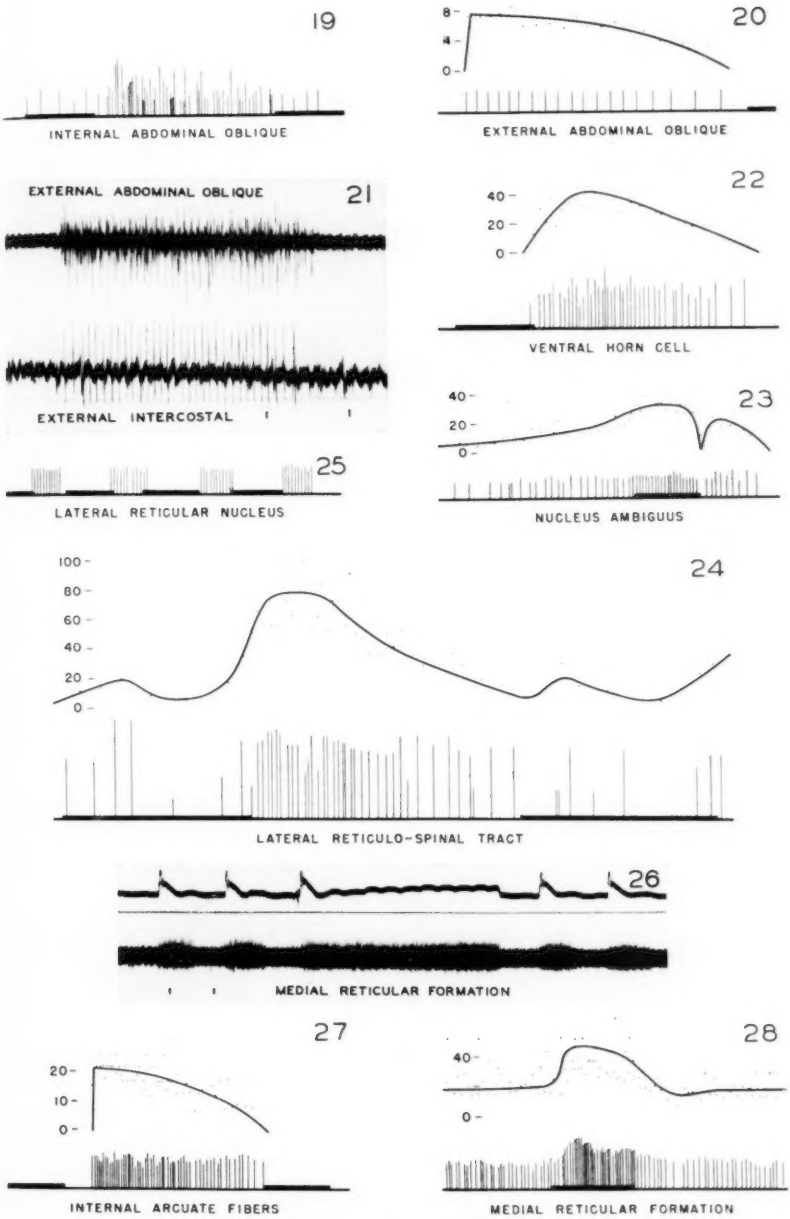
Figs. 22 and 23. Electrical discharges and their frequency curves of a ventral horn cell and of the nucleus ambiguus during the phase of expiration and in the latter during the phase of inspiration as well. For explanation see page 623.

Fig. 24. Electrical discharges and their frequency curves of the lateral reticulospinal tract showing activity during both expiratory and inspiratory phases.

Fig. 25. Steady state electrical discharges of the lateral reticular nucleus during expiratory activity.

Fig. 26. Steady state fusillades of expiratory activity in the medial reticular formation with tracheal pressure record.

Figs. 27 and 28. Electrical discharges and their frequency curves from the internal arcuate region and the reticular formation. The rapidly augmenting type of discharge from the medial reticular formation is atypical during the phase of inspiration.


19
INTERNAL ABDOMINAL OBLIQUE
8
4
0
20
EXTERNAL ABDOMINAL OBLIQUE
EXTERNAL ABDOMINAL OBLIQUE
21
EXTERNAL INTERCOSTAL
40
20
0
22
VENTRAL HORN CELL
40
20
0
23
NUCLEUS AMBIGUUS
25
LATERAL RETICULAR NUCLEUS
100
80
60
40
20
0
24
LATERAL RETICULO-SPINAL TRACT
26
MEDIAL RETICULAR FORMATION
27
20
10
0
INTERNAL ARCUATE FIBERS
28
40
0
MEDIAL RETICULAR FORMATION

Figs. 19–27

reflex arcs, out to the primary sensory neuron and, therefore, presumably to the peripheral receptors.

It now remains to determine how consistently the slowly augmenting type prevails along the inspiratory arcs and how uniformly the rapidly augmenting and steady state types are found in the expiratory phase of breathing. That question is answered by an analysis of 125 observations from 122 locations histologically determined. Of these 88 were slowly augmenting discharges of which 87 were inspiratory. One slowly augmenting type was found in the internal arcuates during the expiratory phase. There were 22 of the rapidly augmenting type, 19 of which occurred in the expiratory phase and 3 in the inspiratory phase. One of these exceptions is illustrated in the unicellular discharge occurring in the medial reticular formation (see fig. 28). The other two exceptions occur in the internal arcuate fibers. Of the steady state type there were 15, all of which occurred during the expiratory phase of breathing. The answer to our question regarding the incidence of the three primary pattern types, therefore, is clear. The slowly augmenting potentials are typically inspiratory. The rapidly augmenting and the steady state patterns are typically expiratory.

The relative incidence of respiratory potentials at various neural stations is worthy of note. Had it been possible to collect more data, the statistical value of this analysis would have been considerably enhanced, but the work did not progress without its disappointments. In many experiments we were unable to find any respiratory potentials whatever, even with the most searching efforts. Such scarcity of potentials would seem to indicate that relatively few cells are needed in the medulla to activate enough ventral horn cells to carry on eupneic breathing (see Gesell, 1939b). Other experiments, in which good respiratory discharges were encountered, were failures due to the inability to find the electrolytic lesions which were placed for the histological identification of the source of the potentials. To be of value the lesions must be small.

The high incidence of potentials in the reticular nuclei and reticular formations and in the internal arcuates, regions generally regarded as important in the control of breathing, is worthy of note. These structures are relatively large and easily contacted and for that reason might also account for high incidence of respiratory potentials. Another important structure is the parafascicular gray which must undoubtedly discharge in every respiratory act and yet we had very few of these highly desirable potentials until we concentrated our soundings in the region of this relatively small structure. (Findings other than those on the parafascicular gray were obtained in a systematic symmetrical system of soundings and may be evaluated accordingly.) A relatively high incidence of expiratory potentials in the ventral horns was rather unexpected and may be due only

to freak chance, for if there is one point that seems to be established, it is the relatively high incidence of inspiratory as compared with expiratory potentials. In this particular series the ratio stands 90 to 34 which is in close agreement with earlier electrical recordings of respiratory muscles by Gesell (1936a). The inference that eupneic breathing is primarily an inspiratory phenomenon is, thereby, strengthened.

Discussion. We have already suggested that muscular activity may be expected to set up discharges in the outlying proprioceptive end organs proportionate to the distorting forces. That assumption will account for the similarity of electrical discharges throughout the motor and sensory portions of the respiratory arcs but it leaves more pressing problems unsolved. What, for example, is the basic mechanism producing the slowly augmenting inspiratory contraction? If such contractions can originate within the center itself without the intervention of afferent signals (Gesell, 1940) a second question is raised. What are the effects of a slowly augmenting stream of proprioceptive signals upon the slowly augmenting central discharge?

SUMMARY AND CONCLUSIONS

In an earlier publication we have reported results identifying the neurohistological elements of the brain stem which give rise to periodic potentials in phase with breathing in the dog. These potentials were recorded with the oscillograph and bipolar electrodes.

The present work follows on the former and is concerned with three details of these discharges: 1, the characteristics of the discharge pattern; 2, the correlation between discharge pattern and the phase of respiration; and 3, the correlation between discharge pattern and the histological source of the potential.

The results lead to the following conclusions:

1. There are three main types of respiratory discharges: A, the slowly augmenting type in which activity builds up slowly and subsides abruptly; B, the rapidly augmenting type in which activity builds up suddenly and subsides slowly; and C, the steady state type in which activity is periodically maintained at a constant level.

2. Inspiratory potentials are almost exclusively of the slowly augmenting type, while expiratory potentials are of the rapidly augmenting and steady state types.

3. This relation between the phase of respiration and discharge pattern is found at all points along the respiratory arc from respiratory muscles through the central nervous system and back along the sensory nerves.

Factors leading to this parallelism of activity in serially linked stations of the respiratory mechanism have been suggested.

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